National Institute on Alcohol Abuse and Alcoholism

RESEARCH
Monograph-9

NIDA Addiction Research Center

Biological/Genetic Factors in Alcoholism

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration

Research Monograph No. 9

BIOLOGICAL / GENETIC FACTORS IN ALCOHOLISM

Sponsored by:

National Institute on Alcohol Abuse and Alcoholism
American College of Neuropsychopharmacology
University of Connecticut Alcohol Research Center

Edited by:

Victor M. Hesselbrock,
Edward G. Shaskan, and
Roger E. Meyer

NATIONAL INSTITUTES OF HEALTH
NIH LIBRARY

SEP 26 2007

BLDG 10, 10 CENTER DR
BETHESDA, MD. 20892-1150

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES

Public Health Service
Alcohol, Drug Abuse, and Mental Health Administration

National Institute on Alcohol Abuse and Alcoholism
5600 Fishers Lane
Rockville, Maryland 20857

This publication is the product of a research workshop on biological and genetic factors in alcoholism supported under National Research Center Grant No. AA03510 awarded by the National Institute on Alcohol Abuse and Alcoholism to the University of Connecticut Health Center. The presentations herein are those of the listed authors and may not necessarily reflect the opinions, official policy, or position of the National Institute on Alcohol Abuse and Alcoholism, the Alcohol, Drug Abuse, and Mental Health Administration, the Public Health Service, or the U.S. Department of Health and Human Services.

The National Institute on Alcohol Abuse and Alcoholism has obtained permission from the copyright holders to reproduce the figures and tables on pp. 42, 58, 59, 60, 62, 63, 81, 82, 83, and 84. Further reproduction of these materials is prohibited without specific permission of the copyright holder. Adapted tables of copyrighted materials may be copyrighted; thus before reprinting, readers are advised to determine the copyright status or to secure permission of the copyright holder. All other material contained in the monograph, except quoted passages from copyrighted sources, is in the public domain and may be used and reprinted without permission. Citation of the source is appreciated.

The U.S. Government does not endorse or favor any specific commercial product or commodity. Trade or proprietary names appearing in this publication are used only because they are considered essential in the context of the studies reported herein.

Library of Congress Catalog Card Number: 82-600526

DHHS Publication No. (ADM)83-1199
Printed 1983

For sale by the Superintendent of Documents, U.S. Government Printing Office, Washington, D.C. 20402

FOREWORD

This volume is another in the series of the National Institute on Alcohol Abuse and Alcoholism's research monographs. The series sets forth current information on a number of topics relevant to alcohol abuse and alcoholism as reported through workshops in the research, prevention, and treatment areas, as well as through state-of-the-art reviews on selected subjects.

The NIAAA has had a longstanding commitment to broad dissemination of information. This monograph series informs researchers, clinicians, program administrators, and other interested persons about significant findings that may be useful in improving alcoholism programs.

Loran D. Archer
Deputy Director
National Institute on Alcohol Abuse
and Alcoholism

NIH LIBRARY

PREFACE

This monograph reports the proceedings of a research workshop cosponsored by the University of Connecticut Alcohol Research Center, the National Institute on Alcohol Abuse and Alcoholism (NIAAA), and the American College of Neuropsychopharmacology (ACNP). The workshop was held in December 1980 on the occasion of the annual meeting of the ACNP.

The Alcohol Research Center is one of nine national alcohol research centers funded by NIAAA. Each center focuses on a central theme of importance to the study of alcoholism, ranging from the neurophysiological effects of alcohol consumption to the study of factors influencing drinking patterns and trends and the prevention of alcohol problems. In addition to their primary mission of developing new knowledge through original research in their chosen areas within the field of alcoholism, the centers are responsible for disseminating that knowledge broadly across scientific and lay communities. In this connection, the centers have joined the NIAAA in organizing and holding workshops and conferences on topics close to their specialty areas. The proceedings in this volume represent one of these collaborative activities.

The purpose of the workshop was to review the biological and genetic factors associated with the etiology of alcoholism. There are a number of studies that suggest a biological-genetic basis for alcoholism in terms of increased susceptibility for developing alcoholism, especially within the context of specific environmental conditions. This monograph presents discussions of many of the methodological issues that have concerned researchers in the field. It is hoped that this monograph will be of value to investigators who are currently working in this area or are planning to do so in the future. In addition, it is hoped that these proceedings will be generally informative to the interested reader.

Albert A. Pawlowski, Ph.D.
Chief
National Research Centers Branch
Division of Extramural Research
National Institute on Alcohol Abuse
and Alcoholism

CONFERENCE PARTICIPANTS

John R. Dannecker, Jr., M.S.
Alcohol Research Center
Department of Psychiatry
University of Connecticut
Health Center
Farmington, CT

Richard A. Deitrich, Ph.D.
Alcohol Research Center
Department of Pharmacology
University of Colorado Health
Sciences Center
Denver, CO

Samuel Eiduson, Ph.D.
Alcohol Research Center
Department of Biological Chemistry
UCLA Medical School
Los Angeles, CA

Victor M. Hesselbrock, Ph.D.
Alcohol Research Center
Department of Psychiatry
University of Connecticut
Health Center
Farmington, CT

Charles S. Lieber, M.D.
Alcohol Research and Treatment
Center
Bronx VA Medical Center and
Mt. Sinai School of Medicine (CUNY)
New York, NY

Roger E. Meyer, M.D.
Alcohol Research Center
Department of Psychiatry
University of Connecticut
Health Center
Farmington, CT

Lars Oreland, M.D.
Department of Pharmacology
University of Umea
Umea, Sweden

Dennis R. Petersen, Ph.D.
Alcohol Research Center
University of Colorado School
of Pharmacy
Denver, CO

Theodore Reich, M.D.
Alcohol Research Center
Department of Psychiatry
Washington University School of
Medicine and the
Jewish Hospital of St. Louis
St. Louis, MO

John Rice, Ph.D.
Alcohol Research Center
Department of Psychiatry
Washington University School of
Medicine and the
Jewish Hospital of St. Louis
St. Louis, MO

Marc A. Schuckit, M.D.
Department of Psychiatry
VA Medical Center
San Diego, CA

Edward G. Shaskan, Ph.D.
Alcohol Research Center
Department of Psychiatry
University of Connecticut
Health Center
Farmington, CT

John L. Sullivan, M.D.
Neuropsychopharmacology Laboratory
VA Medical Center and
Duke University Medical Center
Durham, NC

Table of Contents

Page

Foreword iii
Preface iv
Conference Participants v
Introduction
Victor M. Hesselbrock, Edward G. Shaskan, and Roger E. Meyer viii

Models for the Familial Transmission of Alcoholism: Path Analysis and Multifactorial Segregation Analysis
John Rice, Theodore Reich, and C. R. Cloninger 1

Acetaldehyde and Alcoholism: Methodology
Marc A. Schuckit 23

Breath Acetaldehyde: Highly Sensitive Evaluation of Endogenous and Exogenous Ethanol Metabolism
John R. Dannecker, Jr., Edward G. Shaskan, and Roger E. Meyer 49

Elevation of Acetaldehyde Levels After Chronic Alcohol Consumption: Pathogenesis and Pathologic Consequences
Charles S. Lieber, Enrique Baraona, Ellen R. Gordon, Pekka Jauhonen, M. E. Lebsack, and Pekka Pikkarainen 67

Brain Acetaldehyde Metabolism During Ethanol Consumption
Dennis R. Petersen, V. Gene Erwin, and Richard A. Deitrich 93

Assessment of Platelet Monoamine Oxidase Activity as a Risk Factor in Epidemiologic Research
Edward G. Shaskan 101

Why Do Alcoholics Have Low Platelet Monoamine Oxidase Activity?
Lars Oreland 119

Predictive Value of Platelet Monoamine Oxidase Activity in the Treatment of Depressed Alcoholics With Lithium
John L. Sullivan, Paula D. Sullivan, Jonathan Davidson, C. Edward Coffey, Steven Mahorney, Ronald J. Taska, and Jesse O. Cavenar 131

Effect of Maternal Alcohol Ingestion on Developing Rat Brain MAO
Samuel Eiduson and Regina Groshong 145

Summary
Victor M. Hesselbrock, Edward G. Shaskan, and Roger E. Meyer 159

NIH LIBRARY

Introduction

Victor M. Hesselbrock, Edward G. Shaskan, and Roger E. Meyer

Although alcoholism is a strongly familial disorder, its distribution within a population cannot be explained by either genetic or environmental factors alone (Cloninger et al. 1981). A variety of data from twin, adoptee, and family studies suggests the heritability of alcoholism. It is estimated that if a person has a first degree relative who is an alcoholic, then that person has a 60 percent chance of developing an alcohol-related illness. One question that occurs repeatedly in considering genetic factors in alcoholism is the issue of a general genetic predisposition to psychopathology (of which alcoholism is one possible outcome) versus a specific genetic vulnerability for alcoholism.

Schuckit and associates (1979) examined blood acetaldehyde levels after alcohol ingestion in family members of alcoholics, and their findings are consistent with a specific genetic vulnerability. Animal data, in which strain differences are associated with differences in ethanol preference and consumption, also suggest a specific genetic factor. Consistent research findings that alcoholic individuals and their family members frequently share the characteristic of low levels of platelet monoamine oxidase support the notion of more generalized vulnerability for psychopathology, with environmental factors determining specific manifestations of psychopathology. Application of newer quantitative genetic models to previously collected family data on alcoholism (Cloninger et al. 1979) has produced findings that tend to support the notion of a generalized vulnerability for alcoholism. Unpublished research at the University of Connecticut Alcohol Research Center has found high rates of alcoholism in families of patients being treated for alcoholism and, paradoxically, equally high rates of alcoholism in families of patients with other kinds of psychiatric disorders.

A second question is the definition of alcoholism within the context of its heritability. This issue also has plagued genetic studies of schizophrenia and affective disorder. Are there genetically distinct subtypes of alcoholism? To what degree can these subtypes be defined by associated psychopathology? Do the various consequences of alcoholism (liver pathology, central nervous system pathology, and psychosocial deterioration) constitute discrete, genetically definable populations? Clearly, central nervous system pathology, liver pathology, and other consequences of alcohol abuse do not occur in all persons diagnosed as having alcoholism. Is there a relationship between family pedigree of alcoholism and prognosis, as has been suggested by Francis and associates (1980)? The notion of a depressive spectrum disorder (Winokur et al. 1975) suggests that temperament and personality variables are inherited and at some level may predispose some individuals to alcoholism in this society. Is it possible that alcoholism may also be a spectrum disease?

Finally, relative to the work that follows, the convergence of research on platelet monoamine oxidase and acetaldehyde brings back the intriguing and unproven hypothesis of a common mechanism of physical dependence related to alcohol addiction and opioid addiction. Davis and Walsh (1970) have suggested that acetaldehyde and dopamine may form tetrahydroisoquinolines (TIQs), and that acetaldehyde and serotonin may form betacarbolines. These consequent molecules structurally resemble opioid alkaloids. Although pharmacologic cross-tolerance is not shared, other etiologic mechanisms may exist.

The distinction between a biological marker and a biological risk factor is also important when examining the question of gene-environment interaction in alcoholism research. The emphasis of this monograph is on the latter concept. In general, a biological marker for alcoholism is considered to be any biological indicator of alcohol abuse or dependence resulting from such abuse or dependence. The identification of a biological marker that is both sensitive and specific for alcoholism would be of great value in making a differential diagnosis. To date, however, several biological variables are sensitive to alcohol abuse (e.g., SGOT, GGTP, MCV), but are not necessarily specific to alcohol abuse. Nor do abnormal values on these biological variables in individuals not affected with alcoholism necessarily predispose those individuals to alcoholism.

A biological "risk" factor, however, is a variable that exists prior to the onset of the condition and, therefore, is distinguished from a similar outcome of the disease process. Risk factors are frequently nonspecific independent variables that combine in multifactorial sets to predispose or precipitate disease in a population. It should be remembered, however, that a risk factor may or may not be genetically transmitted. Some criteria necessary for a biological

NIH LIBRARY

variable to be considered a genetically transmitted risk factor (Rieder and Gershon 1978) include the following:

1. The characteristic must be associated with an increased likelihood of developing the disorder.
2. The characteristic must be heritable and not a secondary effect of the disorder.
3. The characteristic must be observable or evocable in both the well state and the ill state. Because the factor indicates a predisposition to the disorder and not part of the disorder, it is expected some well relatives and recovered relatives would have the characteristic.
4. The transmission of both the characteristic and the disorder must be related within pedigrees.

The focus of this symposium is on two variables that have received considerable attention in the literature as putative biological risk factors for alcoholism: monoamine oxidase (MAO) and acetaldehyde (AcH). There is evidence that MAO activity represents a stable genetic trait among alcoholic and nonalcoholic cohorts (see Oreland, this volume). Levels of AcH, the first metabolic product of ethanol metabolism, also are genetically controlled, depending on the activities of at least two enzymes (alcohol and acetaldehyde dehydrogenases); however, a complex assortment of dietary factors, diffusional barriers, and carrier proteins could confound heritable control with environmental factors.

Mean platelet MAO activity and blood and breath AcH for different samples of alcoholics, including cohorts of individuals with family histories positive for alcoholism, have been reported to differ from nonalcoholic case control groups. Unfortunately, the study designs used frequently fail to address the issue of whether the independent variables, as putative predisposing factors, are really outcome factors of a complex disease and its associated process. Similarly, the issue of heritability is often not considered—hence, the "and/or" in the title of this monograph. The purpose of the workshop and its resulting monograph is to identify issues of study design from behavioral genetics and epidemiology that can be used within the milieu of practical assessments of these two biological variables (and that may also be applicable to other biological variables) in either human populations or animal models.

To accomplish the purpose of integrating these areas in relation to alcoholism, the monograph begins with a paper by Rice and coworkers that presents the multifactorial model of inheritance.

Then papers by Schuckit, Dannecker and coworkers, Lieber and coworkers, and Petersen and coworkers focus on the pathogenesis of alcoholism and an assessment of acetaldehyde following ethanol consumption. Possible effects of genetic and environmental factors on acetaldehyde levels in blood and brain are also considered. Data from human and animal studies are presented. The group of papers by Shaskan, Oreland, and Sullivan and coworkers examine possible reasons for the low level of MAO activity often found in alcoholics and the implications of this finding for treatment. The final paper, by Eiduson and Groshong, examines the effects of maternal alcohol ingestion on brain MAO activity in developing rats. All papers in this final series provide insight into the potential usefulness of MAO activity as a risk factor in alcoholism research.

It is hoped that this focus on biological factors associated with the heritability of alcoholism, as well as the presentation of mathematical models from behavioral genetics, will be of value to researchers attempting to examine genetic and environmental factors in the etiology of alcohol abuse.

Most of the participants in this workshop are from NIAAA-funded alcohol research centers, including the University of Connecticut, Washington University in St. Louis, the University of California at Los Angeles, the University of Colorado, and the Mt. Sinai School of Medicine in New York City. Participants were also invited from the Veterans Administration Medical Center in San Diego, the Duke University Medical School, and the University of Umea, Sweden. We would like to thank the panel members for their participation. In particular, we wish to thank Oakley Ray and the American College of Neuropsychopharmacology for their support.

NIH LIBRARY

References

Cloninger, C.R.; Bohman, M.; and Sigvardsson, S. Inheritance of alcohol abuse. *Arch Gen Psychiatry*, 38:861-871, 1981.

Cloninger, C.R.; Reich, T.; and Wetzel, R. Alcoholism and affective disorders: Familial and genetic models. In: Goodwin, D., and Erickson, C., eds. *Alcoholism and Affective Disorders*. New York: S.P. Medical & Scientific Books, 1979.

Davis, V.E., and Walsh, M.J. Alcohol, amines, and alkaloids: A possible basis for alcohol addiction. *Science*, 167:1005-1007, 1970.

Francis, R.J.; Timm, S.; and Bucky, S. Studies of familial and nonfamilial alcoholism. *Arch Gen Psychiatry*, 37:564-566, 1980.

Rieder, R.O., and Gershon, E.S. Genetic strategies in biological psychiatry. *Arch Gen Psychiatry*, 35:866-873, 1978.

Schuckit, M., and Rayses, V. Ethanol ingestion differences in blood acetaldehyde concentration in relatives of alcoholics and controls. *Science*, 203:54-55, 1979.

Winokur, G.; Cadoret, R.; Baker, M.; and Dorzab, J. Depressive spectrum disease vs. pure depressive disease. *Br J Psychiatry*, 127:75-77, 1975.

Models for the Familial Transmission of Alcoholism: Path Analysis and Multifactorial Segregation Analysis*

John Rice, Theodore Reich, and
C. R. Cloninger

Summary

The multifactorial model of inheritance for a qualitative trait is described in detail. This model postulates an underlying continuous liability scale with one or more threshold values that determine phenotypic manifestations of a trait. With a single threshold, an individual is classified as "affected" or "unaffected," and with multiple thresholds, individuals may be classified according to severity or to sex. Such traits arise when the underlying process is continuous, but the phenotypic effects can be measured only within certain ranges. Similarity between relatives is quantified by their correlation in liability using the tetrachoric correlation coefficient.

A description of the use of path analysis to model the sources of familial resemblance is also given. Path analysis uses systems of regression equations to describe the determination of one variable by other variables in a model. The path model is used to interpret the correlations in liability between relatives and to provide a vehicle for hypothesis testing.

NIH LIBRARY

Note: Figures appear at end of paper.

*Supported in part by U.S. Public Health Service grants AA-03539, MH-31302, MH-25430, and MH-00048 (CRC).

Procedures are given for estimating the correlations or the path parameters from family data collected using the proband method. An application of these methods to a preliminary analysis of alcoholism data is described, and some future directions for this type of analysis are indicated.

Introduction

Most medical and psychiatric illnesses tend to cluster within families. Indeed, the best predictor of illness can be the presence of illness in other family members, even when compared with the premorbid characteristics of the individuals themselves. However, most disorders do not follow classical Mendelian models of genetic transmission. Such deterministic patterns of inheritance are unlikely to be found either when clinical manifestations are heterogeneous or when environmental factors make a significant contribution to the development of an illness. Rather, one must formulate models that include both genetic and nongenetic types of variation and that allow for the subtyping of affected individuals to resolve possible heterogeneities. Although genetic effects have been shown to be important for many psychiatric disorders, the extent to which these effects operate and the ways in which they interact with the environment to produce an illness are unknown.

Genetic epidemiology (Morton and Chung 1978; Sing and Skolnick 1979) is a new and burgeoning field of genetics that addresses the inheritance of complex traits in man. In their review of quantitative inheritance, Morton and Rao (1978) wrote: "Human geneticists tend to hug the coasts of Mendelian traits without venturing into the deep waters of complex inheritance." In genetic epidemiology, emphasis is placed on the analysis of traits of biomedical significance using family data. Inheritance is used in its broad sense to include both genetic and cultural transmission, and a primary goal is to unravel the sources of familial resemblance. Family studies offer a valuable tool for investigating traits for which familial resemblance is high, but for which biological or environmental correlates are not yet well understood.

In psychiatry, family studies have been advocated as a tool for defining and validating nosological categories (Goodwin and Guze 1979) and offer a strategy for establishing biological markers (Rieder and Gershon 1978). Moreover, the last decade has witnessed much progress in the development of standardized diagnostic instruments, and a number of large-scale family studies that utilize these instruments are nearing completion. Accordingly, the methods described below are likely to play an important future role in formulating and testing nosological hypotheses, establishing the mode of transmission for certain disorders, and identifying familial and nonfamilial environments relevant to etiology.

In this chapter we shall describe the multifactorial model of inheritance for threshold traits, path analysis, segregation analysis, and the statistical methods used in applying these techniques to family data. We shall also indicate some extensions of these techniques, which our group, as well as others, are currently developing.

NIH LIBRARY

Current Methods

The Threshold Concept

The use of threshold models has become increasingly popular in human genetics since Crittenden (1961) and Falconer (1965, 1967) presented the multifactorial model based on an underlying liability scale. It is assumed that all relevant genetic and nongenetic sources of variation can be combined into a single continuous variable termed "the liability to develop the disorder." The liability distribution is dichotomized by a threshold value, so that an individual is "affected" with the disorder if his score is above the threshold and unaffected otherwise. This model has been discussed by Reich et al. (1972, 1975), Curnow and Smith (1975), and Boyle and Elston (1979), and was first applied in psychiatry by Gottesman and Shields (1967) for schizophrenia.

Figure 1 illustrates the basic threshold model for a trait with phenotypes affected and unaffected. An individual whose liability score is above the threshold value S manifests the disorder, and an individual whose score is below the threshold does not. The proportion of affected individuals in the population (K_p) is the area to the right of S and under the probability density of X (i.e., the shaded area in figure 1). The mean liability of affected individuals is denoted by a, and is simply the average score of affected individuals.

It is often instructive to have a concrete example in mind when considering these models, so let us take the trait "tall," where X is height measured in inches, and S equals 6 feet 2 inches. The tables found in Falconer (1965), which give S and a for various values of K_p, are especially helpful in performing these types of calculations. If height is normally distributed with, say, a mean of 5 feet 8 inches and a standard deviation of 3.06 inches, then S is 1.96 standard deviations above the mean, so that 2.5 percent of people would be "tall," and the average height of "tall" people could be computed to be 6 feet 3.15 inches. In practice, the liability distribution is not measured, but only the qualitative phenotypic effects, so that the values of the mean and standard deviation of X may be chosen for convenience.

If a variable X is normally distributed (i.e., the associated probability curve is the usual "bell-shaped" curve), and has mean μ and variance σ^2, we will use the notation $X \sim N(\mu,\sigma^2)$. Thus if X denotes the variable height as above, we write $X \sim N(5'8'', (3.06)^2)$ as a convenient shorthand for the assumption that height is normally distributed with mean 5 feet 8 inches and standard deviation 3.06 inches.

The threshold S as defined above is termed an "abrupt threshold." Alternatively, S may be replaced by the concept of a "risk function" in such a way that the probability of manifesting the disease is an increasing function of the liability score (Edwards 1969; Curnow and Smith 1975). When the risk function is in the form of a normal distribution function, the abrupt threshold and the risk function models are formally equivalent (Curnow and Smith 1975). This removes the somewhat arbitrary division between individuals with similar liability scores who lie on either side of S.

The basic threshold model may be extended in several directions. Reich et al. (1972) suggest the use of multiple thresholds to classify affected individuals, as in figure 2, where individuals whose liability scores are between S_1 and S_2 are assumed to represent clinically less severe cases than those individuals whose liability is above S_2. With multiple thresholds, the hypothesis that putative subforms truly represent a gradation of severity may then be tested (Reich et al. 1979). For example, for affective disorder it may be hypothesized that bipolar affective disorder represents a more severe form of illness than unipolar disorder, or for alcoholism that a particular subclassification represents a gradation as in figure 2. These methods allow a quantification of such hypotheses and, more important, allow an empirical test. The underlying rationale of this approach is that the more severely affected class will have more relatives affected with both types than will the less severely affected class.

In our height example, suppose that individuals whose height is over 6′2″ are "severe" and individuals whose height is between 6′ and 6′2″ are "mild." Then 7.1 percent of people will be mild and 2.5 percent will be severe, with a total of 9.6 percent of people affected with either form.

Different prevalences in males and females have been observed for many psychiatric illnesses, including depression, alcoholism, and antisocial personality (Cloninger et al. 1978), although a systematic approach to evaluate the many conflicting hypotheses invoked to explain these differences has, until recently, been lacking. In figure 3, we assume different liability distributions for males and females, respectively. Since the distribution for females is shifted to the right of that for males, the prevalence of affected females (K_{p2}) is greater than the prevalence of affected males (K_{p1}). The mean difference between the sexes may result either from a systematic cultural bias that differentially applies to one sex or from systematic biological differences between the sexes. The liability distribution of each sex may be standardized separately (Kidd et al. 1973), and a goal in analysis is to evaluate the causes of observed sex differences.

In our height example, we may find that, say, the distributions of height in males and females are given by $X_1' \sim N(5'10'', (3.06)^2)$, $X_2' \sim N(5'6'', (3.06)^2)$, so that if $S = 6'2''$, then 9.6 percent of men and 0.5 percent of women are "tall" by this definition.

Correlations Between Relatives

In the multifactorial model, similarity between relatives is quantified by their correlation in liability. However, the estimation of these correlations is complicated by not observing the liability scores themselves. With a single threshold, pairs of relatives sampled at random may be arranged in a 2x2 table with the marginal distributions taking on the values "affected" and "unaffected." The correlation between the underlying liability distributions may be estimated using the tetrachoric correlation coefficient (Kendall and Stuart 1973). This type of correlation is different from the correlation between the qualitative phenotypes (i.e., the phi

NIH LIBRARY

coefficient computed from the 2x2 table). We will consider in detail the estimation of the underlying correlation using relatives of affected individuals. In a typical medical application, affected individuals (probands) are sampled and then their relatives are studied. Consider the model as in figure 1, where X is normally distributed with mean 0 and standard deviation 1. If the correlation between an individual and a class of relatives is r, then the mean (μ) and variance (σ^2) of relatives of affected individuals are given by

$$\mu = ar \tag{1}$$

$$\sigma^2 = 1 - r^2a(a-S), \tag{2}$$

where a is the mean of the affected individuals as indicated in figure 1. Equation (1) corresponds to the phenomenon of regression toward the mean, where the relatives of affected individuals are less deviant than the probands themselves, with the mean of relatives depending linearly on r. Equation (2) corresponds to the reduction of variability when the range of a correlated variable is restricted.

Under the abrupt threshold model the distribution of liability in the relatives is not normal, but under the risk function model it is (Curnow and Smith 1975). In the former case, the distribution of relatives may be assumed to be normal to yield an approximation (Reich et al. 1972; Mendell and Elston 1974) that gives acceptable accuracy. Using (1) and (2), the proportion of affected relative (K_R) may be calculated from the normal deviate, S_R, obtained from the threshold value S by subtracting the mean of relatives and dividing by their standard deviation:

$$S_R = \frac{S - ar}{\sqrt{1-r^2a(a-S)}} \tag{3}$$

That is, K_R is the area under the standard normal curve to the right of S_R. As noted by Reich et al. (1972), equation (3) may be used to solve for r, yielding:

$$r = \frac{S-S_R[1-(S^2-S_R^2)(1-(S/a))]^{1/2}}{a + S_R^2(a-S)} \tag{4}$$

Thus, if estimates of K_p and K_R are given, then S, a, and S_R may be computed and equation (4) used to obtain an estimate of r.

Continuing with our height example, where the height of an individual is assumed to be $N(5'8'', (3.06)^2)$ and with $S = 6'2''$, suppose that the correlation r between relatives is 0.5. Then using formulas (1) and (2), the mean and standard deviation of relatives of "tall" people can be calculated to be 5 feet 11.6 inches and 2.38, respectively. Accordingly, 16 percent of the relatives of "tall" people would be "tall" in our example. Conversely, if the prevalence K_p for a trait was 2.5 percent, and we observed a prevalence K_R in relatives of 16 percent, we would estimate r to be 0.5.

The following estimation procedure will be important when generalizing to more complex situations. Suppose we sample relatives of probands and want to estimate the proportion K_R of affected relatives. For simplicity, suppose we sample (independently) five relatives, with the first two found to be affected and the next three found to be unaffected. The likelihood L of the sample is $K_R \times K_R \times (1-K_R) \times (1-K_R) \times (1-K_R) = K_R^2(1-K_R)^3$, since K_R is the probability that a relative is affected and $(1-K_R)$ is the probability that he is unaffected. The principal of maximum likelihood states that we estimate K_R by the value that maximizes the above likelihood. It can be shown that in this setting if N relatives are sampled, with n found to be affected, then the likelihood function L is maximized for the value $K_R = n/N$, i.e., the observed proportion is the maximum likelihood estimate of the true proportion. In our example we would estimate K_R to be $2/5 = 20$ percent.

In the present context, K_R is a function of the (unknown) parameters K_p and r and we write $K_R = P(K_p,r)$ to emphasize that these are the parameters of interest. If N_1 relatives are sampled, with n_1 found to be affected, then the likelihood L_1 in terms of K_p and r is simply

$$L_1 = [P(K_p,r)]^{n_1}\,[1-P(K_p,r)]^{N_1-n_1}. \qquad (5)$$

If the value of K_p is known, the maximum likelihood estimate of r is obtained as the r that maximizes L_1. Maximizing a (positive) function is equivalent to maximizing the logarithm of that function, and in practice it is usually more convenient to maximize

$$\log L_1 = n_1 \log [P(K_p,r)] + (N_1-n_1) \log [1-P(K_p,r)]. \qquad (6)$$

The value of $P(K_p,r)$ may be computed using the approximation described above or from numerical integration of the bivariate normal density function, and various computer algorithms are available to search for the value of r that maximizes equation (6). In the above situation with K_p known, r would be estimated as that correlation in liability that would yield a K_R of n_1/N_1.

If a population sample of size N_2 is found to have n_2 individuals affected, then K_p may be estimated in a similar way by maximizing

$$\log L_2 = n_2 \log(K_p) + (N_2-n_2) \log (1-K_p). \qquad (7)$$

NIH LIBRARY

If both a sample of relatives of probands and a population sample are available, then K_p and r may be estimated simultaneously by maximizing $\log L_1 + \log L_2$. In general, the log likelihood of independent samples is the sum of the log likelihoods of the individual samples, so that if various types of relatives, etc., are sampled, the log likelihood is computed for each one, and the sum maximized to estimate the parameters.

Path Analysis

Path analysis (Wright 1921) is a tool for evaluating the interrelationships among variables by analyzing their correlational structure, and has been applied in genetics to determine the causes of familial resemblance. The relationships among the variables of a model are depicted by a path diagram as in figure 4, where each variable is standardized so as to have mean 0 and variance 1. Singleheaded arrows indicated the direct influence of one (an independent) variable on another (the dependent variable) and curved double-headed arrows indicate correlations between independent variables that are otherwise unexplained in the diagram. Path coefficients (standardized partial regression coefficients) are associated with each single-headed arrow and measure the change in the dependent variable when a unit change is made in that independent variable, with all other independent variables held constant. The correlation between any two variables in the diagram may be obtained by tracing all paths connecting them with the use of a simple set of rules (Li 1975). Path analysis provides a comprehensive framework for modeling the familial transmission of complex traits.

The transmission of many complex traits appears to be influenced both by genic factors and by sociocultural factors, and until recently, with the exception of Wright (1931), direct parent-to-offspring transmission of environmental factors (cultural transmission) was precluded in models of familial resemblance. Current path models simultaneously allow for cultural transmission, genetic transmission, assortative mating, and common environment (Cavalli-Sforza and Feldman 1973, 1978; Cloninger et al. 1979*a, b*; Feldman and Cavalli-Sforza 1977; Morton 1974; Rao et al. 1974, 1976; Rice et al. 1978, 1980*a*).

Here, we shall consider the TAU Model With Sex Effect (Rice et al. 1980*b*, 1981*a*), which has been applied to alcoholism data (Reich et al. 1980). It is assumed that the liability distributions X_1' and X_2' for males and females (see figure 3) are normally distributed with $X_i' \sim N(\alpha_i,\sigma_i^2)$. The standardized distributions are denoted without the prime and are given by

$$X_i = (X_i' - \alpha_i)/\sigma_i. \tag{8}$$

The prevalences in males and females are denoted by K_{p1} and K_{p2}, respectively, and are given as the area to the right of $S_i = ((S - \alpha_i)/\sigma_i)$ under the standard normal curve.

It is assumed that the liability scale X_i' may be partitioned into a component T_i', the effects of all factors (both genetic and cultural) that are transmissible from a parent of sex i to an offspring, and a component E_i', the effects of all other factors, with

$$X_i' = \alpha_i + T_i' + E_i'. \tag{9}$$

Standardization of equation (9) yields

$$X_i = t_i T_i + e_i E_i, \tag{10}$$

where t_i and e_i are path coefficients that measure the relative importance of transmissible and nontransmissible factors, respectively.

Transmission from parent to offspring is described by the path equations

$$T_{0i} = \tau_{1i} T_1 + \tau_{2i} T_2 + r_i R_i \tag{11}$$

where T_{0i}, T_1, and T_2 denote the transmissible components of an offspring of sex i, the father and the mother, respectively, and R_i an uncorrelated residual. Assortative mating is described by the path equation

$$X_1 = mX_2 + r_3 R_3, \tag{12}$$

where m is the correlation in liability between mates.

The path coefficients τ_{ij} are a measure of the influence of a parent of sex i on an offspring of sex j. Rice et al. (1980*b*) describe submodels that include maternal effects, daughter effects, and cross-sex effects models as possible sex-specific cultural mechanisms. In the case of polygenic transmission, we would have all τ_{ij}s equal to 1/2.

When $T_1' = T_2' = T$, i.e., the transmissible factors are identical in males and females, we have that $\tau_{11} = \tau_{12} = \tau_{21} = \tau_{22}$. However, it is possible that E_1' and E_2' have different variances so that t_1 and t_2 may be unequal, and the sex-specific parent-offspring correlation will differ.

NIH LIBRARY

Finally, the model allows for correlations c_{ij} between the shared (nontransmissible) environments of rearing (the *E*s) of sibling of sexes i and j.

Accordingly, the general model has a total of the 12 parameters $\theta = \{t_1, t_2, \tau_{11}, \tau_{12}, \tau_{21}, \tau_{22}, c_{11}, c_{12}, c_{22}, K_{p1}, K_{p2}\}$. The sex-specific correlation in liability between any pair of relatives may be expressed in terms of these path parameters (Rice et al. 1981*a*).

If data on pairs of relatives are arranged by the sex of the proband and the sex of the relative, then the path coefficients determine the correlations in liability, which in turn determine the K_R for that type of relative, so that the log likelihood log L of the data is given as a sum of contributions from each class as an equation (6), where each summand log L_i is of the form

$$\log L_i = n_i \log [P_i(\Theta)] + (N_i - n_i) \log [1 - P_i(\Theta)]. \qquad (13)$$

The parameters of the model may be estimated by maximizing log L, and a FORTRAN program XTAU (Rice 1981), available upon request, may be used for this.

Tests of hypotheses involving linear constraints (such as $t_1 = t_2$, $\tau_{11} = \tau_{12} = \tau_{21} = \tau_{22}$, or $c_{11} = c_{12} = c_{22}$) may be done using the likelihood ratio test where –2 times the difference in the log likelihoods of the unconstrained and constrained model is approximately a chi-square distribution whose degrees of freedom are the number of constraints tested.

Segregation Analysis

To study the transmission patterns of illness, it is necessary to sample families rather than individuals, so the special techniques of segregation analysis (Elandt-Johnson 1971) are needed to analyze the distribution of families. These techniques are now standard for analyses using the single major locus model (Elston and Yelverton 1975). For the multifactorial model, we use the term "multifactorial segregation analysis" to indicate that the unit of analysis is the entire family. If a family consists of a father, mother, m male offspring, and n female offspring, it is assumed that the joint distribution in liability is an $(m+n+2)$– variate normal distribution, with each marginal distribution having mean 0 and variance 1. In order to compute the probability of observing a family with specified phenotypes, it is necessary to evaluate the probabilities associated with the multivariate normal distribution where the correlation matrix contains the correlations in liability.

We use an approximation described by Rice et al. (1979) to achieve this. This enables the computation of the probability of observing r affected out of the m males and s affected out of the n females. If N families with m male and n female offspring are sampled, then the distribution of the observations follows the multinominal distribution with the log likelihood $\log L_{mn}$ given by

$$\log L_{mn} = n_1 \log [P_1(\Theta)] + \ldots + n_k \log [P_k(\Theta)] + \text{constant} \qquad (14)$$

when k is the number of possible phenotypic configurations for these families, Θ denotes the correlations in liability, or a set of path parameters that determine these correlations, and the constant does not depend on the parameters of the model. The full log likelihood is obtained by summing over n and m for the various sibship sizes present in the data.

When families are sampled by the proband method, new parameters π_1 and π_2, termed "ascertainment parameters," are introduced. Briefly, π_i is the probability that an affected individual of sex i will be a proband, and may be used to compute the probabilities of a family conditional on its being ascertained. The parameters may then be estimated from the multinominal distribution of ascertained families.

The overall logic of these procedures is depicted in figure 5. If a path model is used, a goodness-of-fit statistic may be computed by fitting the correlational model and performing a likelihood ratio test.

The reader is referred to Rice et al. (1981*b*) for a more detailed description of these methods.

Application to Alcoholism Family Data

The above multifactorial segregation analysis has been applied to a preliminary analysis of 130 families of alcoholic probands (Reich et al. 1980), where first degree relatives and grandfathers of the probands were used in the analysis. In addition, relatives of 32 control probands were used to estimate the population prevalence of current or past alcoholism. The lifetime prevalences in the relatives of controls were found to be 20 percent in male first degree relatives, 4 percent in female first degree relatives, and 5 percent in grandfathers. The magnitudes of the estimated correlations were striking, with the sex-specific parent–offspring correlations ranging from 0.31 to 0.50, the sex-specific sibling correlations ranging from 0.41 to 0.60, a mate correlation of 0.52, and a grandfather–grandson correlation of 0.32.

The path model was fit to the data, and hypotheses that $\tau_{11} = \tau_{12} = \tau_{21} = \tau_{22} = \tau$ and that $c_{11} = c_{12} = c_{22} = c$ were both accepted, and the hypotheses that $t_1 = t_2 = 0$ and that $c = 0$ were rejected. There was a suggestion that $t_1 \neq t_2$ ($\chi^2_1 = 3.6$), which would indicate that women may be more sensitive to environmental factors than men. Definitive conclusions will be possible when the full data set is available for analysis. With $\tau = 0.5$, the proportion of the variability in liability that is transmissible is estimated to be $t_1^2 =$ 74 percent and $t_2^2 =$ 40 percent for males and females, respectively, and the correlation c between the nontransmissible environments of siblings is estimated to be 0.32.

This analysis indicated that the liability for alcoholism is highly heritable, although it does not provide separate estimates for the genetic and environnmental contributions. Goodwin et al. (1973) reported the lifetime prevalence of alcoholism as 18 percent in the adopted-away sons of Danish alcoholic fathers as contrasted with a 5 percent rate in control adoptive families, yielding a correlation of 0.383. Using this correlation, the genetic heritability is estimated to be 51 percent in males and 29 percent in females, and the cultural heritability is estimated to be 13 and 8 percent, respectively. However, Goodwin's study used different criteria for diagnosis and was done in Denmark, so that the comparability of the two data sets is uncertain. This analysis assumes that the correlations in liability are the same in the two populations and that the two sets of diagnostic criteria correspond to two different truncation points (as reflected by the different prevalences).

Discussion

We have focused our attention on one of several approaches to the analysis of family data. Much theoretical work is also being done for the analysis of threshold characters, using the generalized single major locus model and a mixed model allowing for both a single locus and a multifactorial background. These approaches should be viewed as complementary, with each addressing a different aspect of the underlying biology. All these approaches involving statistical models provide a unified framework to formulate and test hypotheses and to make predictions that can be evaluated in subsequent studies. Moreover, models based on liability-threshold concepts seem appropriate for the analysis of psychiatric disorders, since valid scales that grade both affected and unaffected individuals and that reflect clinical observations are not currently available.

Future directions in this type of modeling will enhance the ability to discriminate among models, to predict risk, and to identify individual biological or environmental factors relevant to the etiology of a disorder. One approach will be to allow for a quantitative measurement that is correlated with the underlying liability distribution, so that two measurements are simultaneously analyzed within families (Smith and Mendell 1974). This permits the use of ancillary personality measures, or biological markers such as platelet MAO or acetaldehyde levels, to provide information (in a predictive sense) on an individual's liability score. Alternatively, the ancillary measure may be incorporated into a path analytic model as an index of a particular component of the liability scale (e.g., home environment relevant to the trait). Techniques for evaluating the properties of a putative index are available (Rice et al. 1980*a*), and the next step will be to develop methods for constructing indices from multivariate data.

NIH LIBRARY

Figure 1. **Liability Distribution *X*, With Threshold Values *S*, and Mean of Affected Individuals *a***

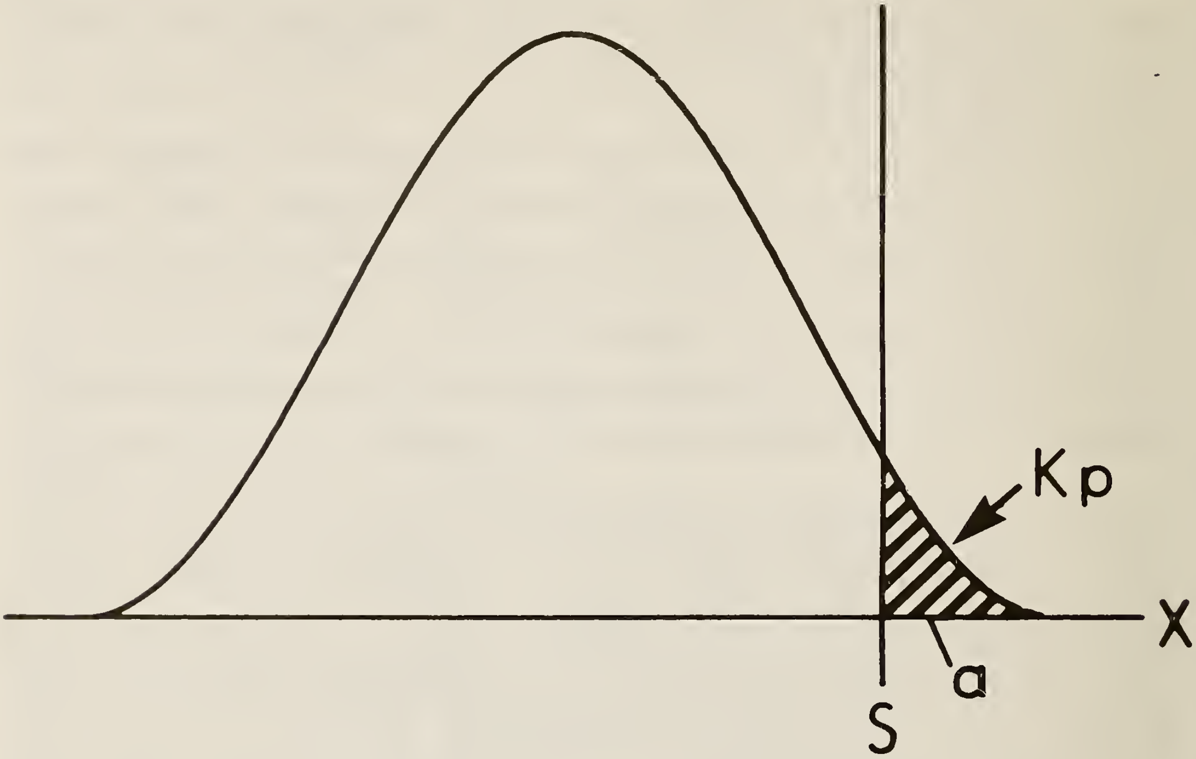


Figure 2. Liability Distribution X for a Trait With Two Thresholds S_1 and S_2 Used to Model Severity of Illness

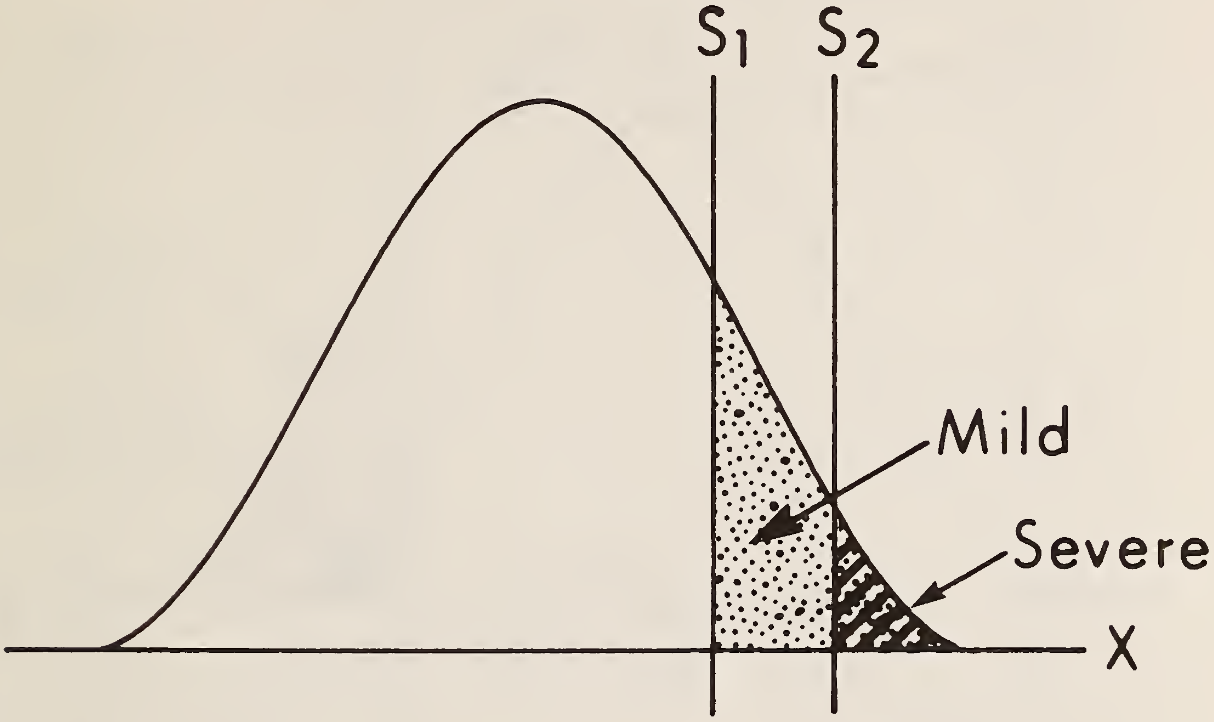


Figure 3. Liability Distributions of Males (1) and Females (2) on a Common Liability Scale X' With a Threshold S', Where Males Have Mean α_1, and Females Have Mean α_2

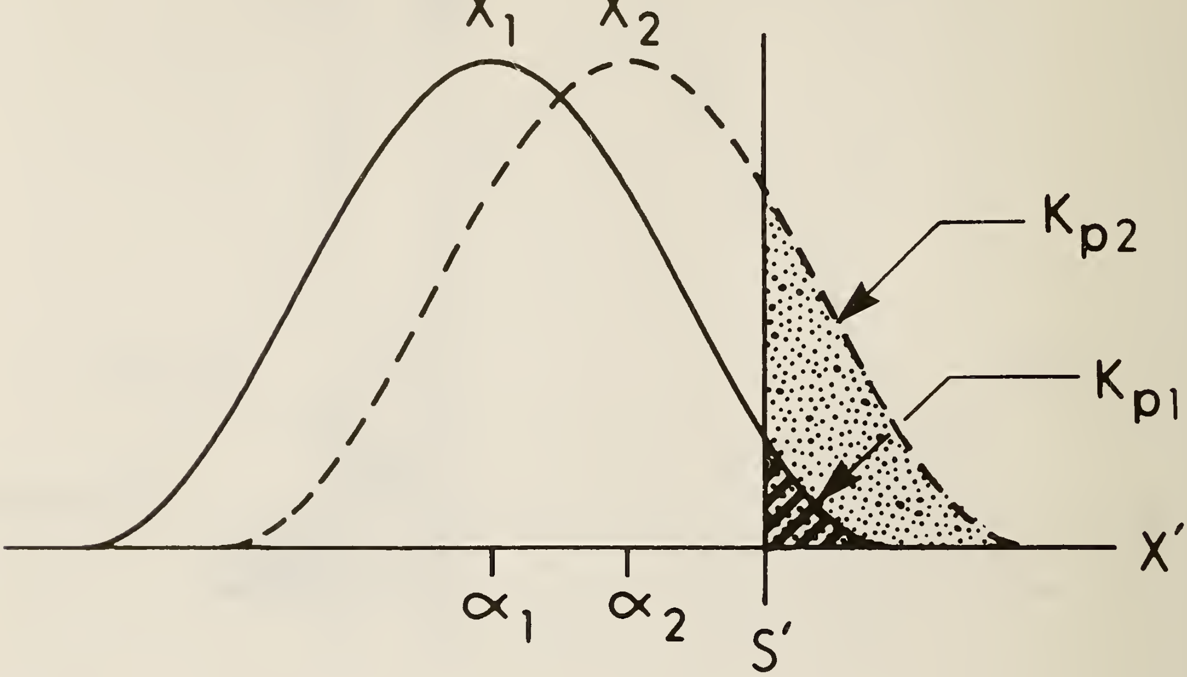


Figure 4. Path Diagram Depicting the Sources of Resemblance Between a Parent and Offspring and Between Siblings

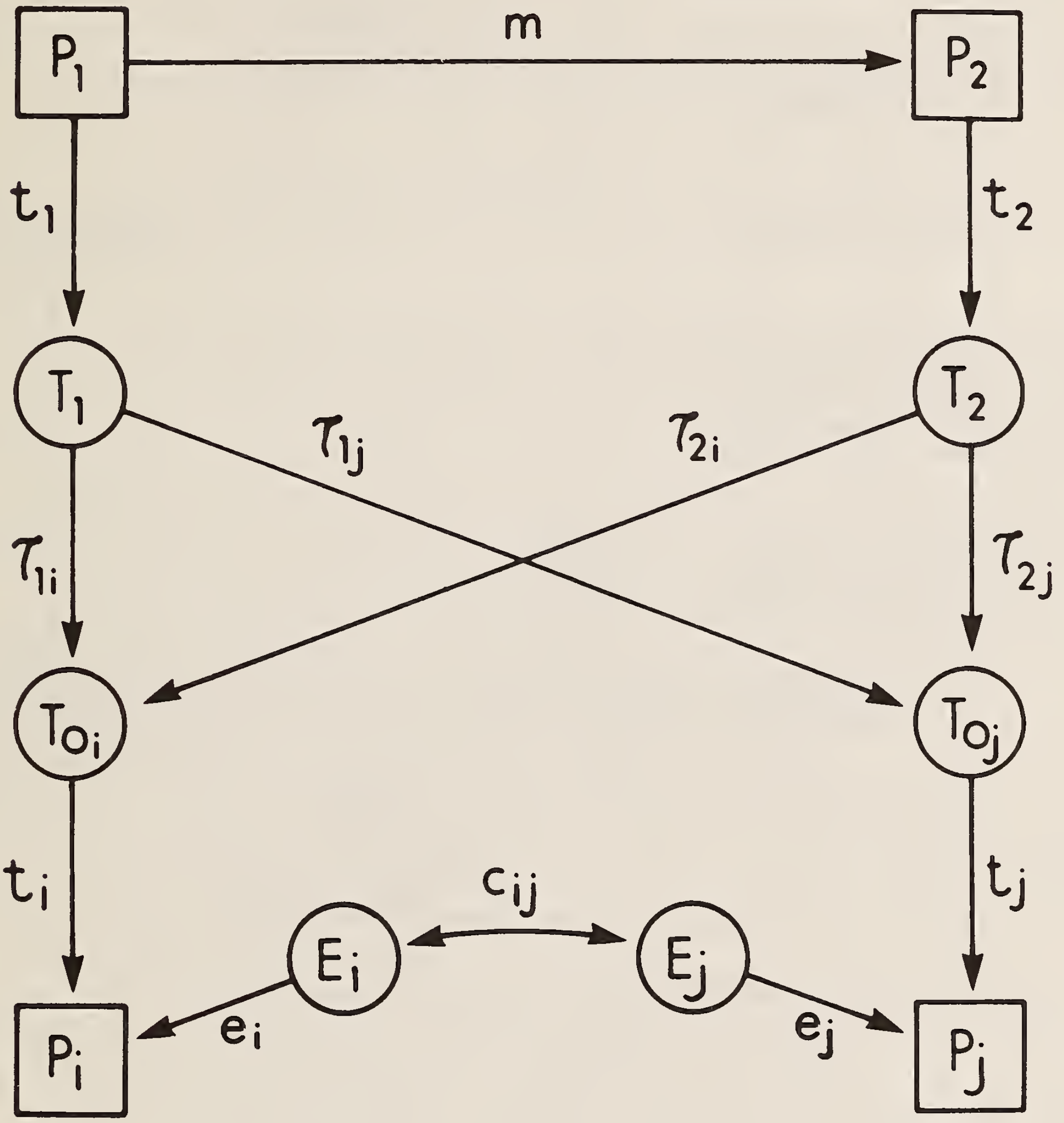
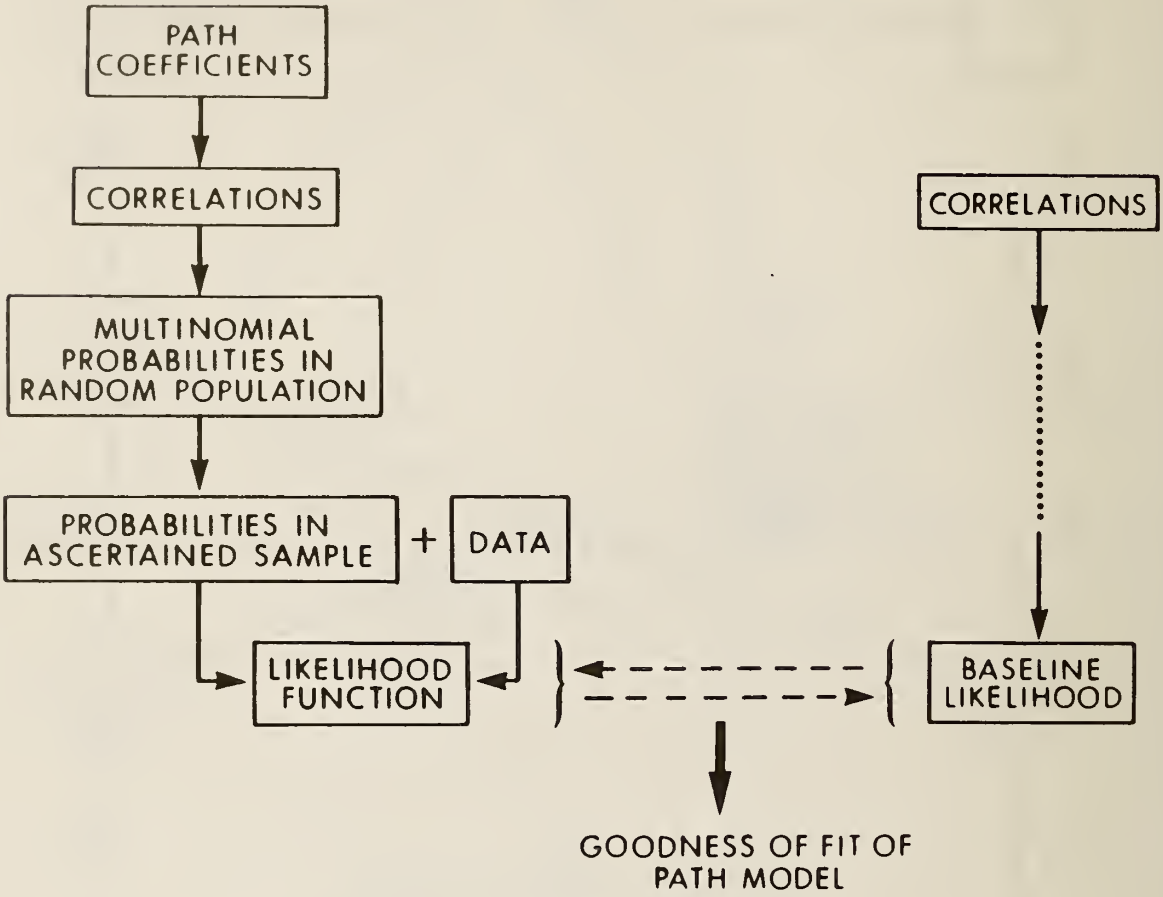


Figure 5. **Logical Sequence Used for Parameter Estimation, Hypothesis Testing, and Assessment of Goodness-of-Fit for Multifactorial Segregation Analysis**


PATH COEFFICIENTS
CORRELATIONS
MULTINOMIAL PROBABILITIES IN RANDOM POPULATION
PROBABILITIES IN ASCERTAINED SAMPLE
+
DATA
LIKELIHOOD FUNCTION
CORRELATIONS
BASELINE LIKELIHOOD
GOODNESS OF FIT OF PATH MODEL

References

Boyle, C.R., and Elston, R.C. Multifactorial genetic models for quantitative traits in humans. *Biometrics*, 35:55-68, 1979.

Cavalli-Sforza, L.L., and Feldman, M.W. Cultural versus biological inheritance: Phenotypic transmission from parents to children (a theory of the effect of parental phenotypes on children's phenotypes). *Am J Human Genet*, 25:613-637, 1973.

Cavalli-Sforza, L.L., and Feldman, M.W. Evolution of continuous variation. III. Joint transmission of genotype, phenotype and environment. *Genetics*, 90:391-425, 1978.

Cloninger, C.R.; Christansen, K.O.; Reich, T.; and Gottesmann, I.I. Implications of sex differences in the prevalences of antisocial personality, alcoholism, and criminality for familial transmission. *Arch Gen Psychiatry*, 35:941-951, 1978.

Cloninger, C.R.; Rice, J.; and Reich, T. Multifactorial inheritance with cultural transmission and assortative mating. II. A general model of combined polygenic and cultural inheritance. *Am J Human Genet*, 31:176-198, 1979*a*.

Cloninger, C.R.; Rice, J.; and Reich, T. Multifactorial inheritance with cultural transmission and assortative mating. III. Family structure and the analysis of separation experiments. *Am J Human Genet*, 31:366-388, 1979*b*.

Crittenden, L.B. An interpretation of familial aggregation based on multiple genetic and environmental factors. *Ann New York Acad Sci*, 91:769-780, 1961.

Curnow, R.N., and Smith, C. Multifactorial models for familial diseases in man. *J Roy Stat Soc Ser A-General*, 138:131-169, 1975.

Edwards, J.H. Familial predisposition in man. *Brit Med Bull*, 25:58-64, 1969.

Elandt-Johnson, R.C. *Probability Models and Statistical Methods in Genetics*. New York: Wiley, 1971.

Elston, R.C., and Yelverton, K.C. General models for segregation analysis. *Am J Human Genet*, 27:31-45, 1975.

Falconer, D.S. The inheritance of liability to certain diseases estimated from the incidence among relatives. *Ann Human Genet*, 29:51-76, 1965.

Falconer, D.S. The inheritance of liability to diseases with variable age of onset, with particular reference to diabetes mellitus. *Ann Human Genet*, 31:1-20, 1967.

Feldman, M.W., and Cavalli-Sforza, L.L. The evolution of continuous variation: II. Complex transmission and assortative mating. *Theor Pop Bio*, 11:161-181, 1977.

Goodwin, D.W.; Schulsinger, F.; Hermansen, L.; Guze, S.; and Winokur, G. Alcohol problems in adoptees raised apart from alcoholic biological parents. *Arch Gen Psychiatry*, 28:238-243, 1973.

Goodwin, D., and Guze, S. *Psychiatric Diagnosis*. 2d edition. New York: Oxford University Press, 1979.

Gottesman, I.I., and Shields, J. A polygenic theory of schizophrenia. *Proc Nat Acad Sci*, 58:199-205, 1967.

Kendall, M.G., and Stuart, A. *The Advanced Theory of Statistics, Vol. 2. Inference and Relationship*. 3d edition. New York: Hafner Publishing, 1973.

Kidd, K.K.; Reich, T.; and Kessler, S. Sex effect and the single gene. The relevance of sex effect in discriminating between genetic hypotheses. *Genetics*, 73:137 (Abstract), 1973.

NIH LIBRARY

Mendell, N.R., and Elston, R.C. Multifactorial qualitative traits: Genetic analysis and prediction of recurrence risks. *Biometrics*, 30:41-57, 1974.

Morton, N.E. Analysis of family resemblance. I. Introduction. *Am J Human Genet*, 26:318-330, 1974.

Morton, N.E., and Chung, C.S. *Genetic Epidemiology*. New York: Academic Press, 1978.

Morton, N.E., and Rao, D.C. Quantitative inheritance in man. *Yearbook Physical Anthro*, 21:12-41, 1978.

Rao, D.C.; Morton, N.E.; and Yee, S. Analysis of family resemblance. II. A linear model for familial correlation. *Am J Human Genet*, 26:331-359, 1974.

Rao, D.C.; Morton, N.E.; and Yee, S. Resolution of cultural and biological inheritance by path analysis. *Am J Human Genet*, 28:228-242, 1976.

Reich, T.; James, J.W.; and Morris, C.A. The use of multiple thresholds in determining the mode of transmission of semi-continuous traits. *Ann Human Genet*, 36:163-184, 1972.

Reich, T.; Cloninger, C.R.; and Guze, S.B. The multifactorial model of disease transmission. I. Description of the model and its use in psychiatry. *Brit J Psychiatry*, 127:1-10, 1975.

Reich, T.; Rice, J.; Cloninger, C.R.; Wette, R.; and James, J. The use of multiple thresholds and segregation analysis in analyzing the phenotypic heterogeneity of multifactorial traits. *Ann Human Genet*, 42:371-390, 1979.

Reich, T.; Rice, J.; Cloninger, C.R.; and Lewis, C. The contribution of affected parents to the pool of affected individuals: Path analysis of the segregation distribution for alcoholism. In: Robins, L.N.; Clayton, P.; and Wing, J., eds. *The Social Consequences of Psychiatric Illness*. New York: Brunner/Mazel.

Rice, J.; Cloninger, C.R.; and Reich, T. Multifactorial inheritance with cultural transmission and assortative mating. I. Description and basic properties of the unitary models. *Am J Human Genet*, 30:618-643, 1978.

Rice, J.; Reich, T.; Cloninger, C.R.; and Wette, R. An approximation to the multivariable normal integral: Its application to multifactorial qualitative traits. *Biometrics*, 35:451-459, 1979.

Rice, J.; Cloninger, C.R.; and Reich, T. Analysis of behavioral traits in the presence of cultural transmission and assortative mating: Applications to IQ and SES. *Behav Genet*, 10:73-92, 1980*a*.

Rice, J.; Cloninger, C.R.; and Reich, T. General causal models for sex differences in the familial transmission of multifactorial traits: An application to human spatial visualizing ability. *Social Biology*, 1980*b*.

Rice, J. GENLIB: A library of computer programs for the genetic analysis of family data. 1981. Available upon request.

Rice, J.; Nichols, P.; and Gottesman, I.I. Assessment of sex differences for qualitative multifactorial traits using path analysis. *Psychiatry Res*, 4:301-312, 1981*a*.

Rice, J.; Reich, T.; Cloninger, C.R.; and Clayton, P. Multifactorial segregation analysis and path analysis. In preparation.

Rieder, R., and Gershon, E. Genetic strategies in biological psychiatry. *Arch Gen Psychiatry*, 35:866-873, 1978.

Sing, C., and Skolnick, M. *Genetic Analysis of Common Diseases: Application to Predictive Factors in Coronary Disease*. New York: Alan Liss, 1979.

Smith, C., and Mendell, N. Recurrence risk from family history and metric traits. *Ann Human Genet*, 37:275, 1974.

Wright, S. Correlation and causation. *J Agri Res*, 20:557-585, 1921.

Wright, S. Statistical methods in biology. *Proc Am Stat Assoc*, 26:155-163, 1931.

NIH LIBRARY

Acetaldehyde and Alcoholism: Methodology

Marc A. Schuckit

Introduction

Acetaldehyde (AcH) has generated great interest in the alcoholism field. Its combination of wide distribution in the body and high levels of biological activity has raised much speculation about its possible role as a mediator of the effects of alcohol and as a causative agent in much of the physical pathology associated with alcoholism.

At first glance, these questions might appear relatively straightforward, but answers have been difficult to obtain. This problem is due in large part to the volatile nature of AcH and its tendency to dissolve in various body fluids and to bind to proteins. Thus, over the years different investigators utilizing different methodologies have arrived at divergent conclusions about the role of AcH in human illness.

The purpose of this paper is to review AcH as it relates to a possible biological predisposition to alcoholism. To do this, I will describe AcH, review its possible physiological actions, examine the data supporting its possible role in the genesis of alcoholism, and present an overview of methodological difficulties in working with this substance. Where appropriate, I will review recent information from our laboratory.

Note: Figures and tables appear at end of paper.

NIH LIBRARY

AcH Chemistry

Acetaldehyde, CH_3CHO, has a molecular weight of 44 and is the second homolog of the class of aliphatic aldehydes starting with formaldehyde. AcH has a boiling point of 20.8°C, which makes it volatile at body temperature. It is soluble in water, organic solvents, and lipids (the latter property exceeding that of ethanol), making it widely distributed in body tissues and fluids.

This substance is believed to be at least 10 to 20 times more toxic than ethanol, with a hypnotic dose one-thirty-fifth of alcohol (Truitt 1971). Combined, all of these properties make acetaldehyde difficult to work with and measure. An additional problem is that dilute solutions keep well for only 2 weeks and that pure solutions slowly polymerize to paraldehyde or metaldehyde and therefore must be distilled before use (Lindros 1978).

There are many routes of AcH production. The major production occurs through alcohol dehydrogenase (ADH) metabolism of ethanol in the cell cytoplasm, especially in the liver (Eriksson 1977*a*; Truitt 1971; von Wartburg 1979). There are at least five and perhaps as many as eight phenotypes of this enzyme that are under genetic control from three autosomal gene loci, two of which have two alleles (Li et al. 1977; von Wartburg 1979). Most Orientals and between 5 and 20 percent of Caucasians have an atypical form of ADH that metabolizes ethanol to AcH more quickly. Other mechanisms of AcH production in the body involve catalase and the microsomal ethanol oxidizing system (MEOS) (Lieber 1978; von Wartburg 1979). The body ordinarily has a very low or nonexistent level of AcH in the absence of an ethanol load, even in the presence of small amounts of AcH produced by cigarette smoke (Horton and Guerin 1974; Lindros 1978).

Acetaldehyde is metabolized by many different routes to acetate (von Wartburg 1979). The major pathway involves aldehyde dehydrogenase (ALDH), the most important and most highly active form of which occurs in mitochondria in the liver (Eriksson 1977*b*; Lindros 1978; Truitt 1971). This most active enzyme has a K_m of less than 1 micromolar/l (μM/l), and like all forms of ALDH, it requires NAD in order to function (Truitt 1971). Aldehyde dehydrogenase (ALDH), of much lesser significance to the metabolism of ethanol, is also present in red blood cells (RBCs), the kidneys, adrenals, gonads, brain, uterus, and small intestine (Deitrich 1966).

There are additional mechanisms for destruction of AcH, but they have lower levels of specificity and less affinity for AcH. These include a condensation reaction with primary amines to form a Shiff base, through the actions of aldolases, the formation of hemiacetals and mercaptohemiacetyls, and through the actions of aldehyde or xanthine oxidases (Lindros 1978; von Wartburg 1979). Small amounts of AcH are excreted also directly in the urine or into the air, the latter action being important in an alternate mechanism for determining body AcH levels, as is discussed later (Truitt 1971).

No matter what methodology is used to analyze AcH in body fluids, it must be recognized that only about 5 percent of the substance produced in the liver is actually released into the blood stream (Lindros 1978). Therefore, blood, brain, and breath AcH are only a poor approximation of what is happening in the liver. The actual blood levels of AcH following ethanol intake are probably quite low—usually in the 1 μg/ml to 2 μg/ml range (about 20 μMol/l to 45 μMol/l)—levels with blood ethanol concentrations in the range of 50 mg/dl to 200 mg/dl (about 11 mM/l to 44 mM/l) (Lindros 1978). The levels reported follow acute ethanol administration and might be different if studies were carried out after chronic intake (Truitt 1971).

No matter what the absolute values, some generalizations can be made. Repeated measures on one individual over time tend to be fairly consistent (Freund and O'Hollaren 1965). Human AcH levels tend to fluctuate throughout 3 to 5 hours of drinking, a finding that might relate to coupling of AcH oxidation with pyruvate-lactate conversion (Majchrowicz and Mendelson 1970; Ridge 1963; Truitt and Walsh 1971). These fluctuating levels, however, appear to rise after the blood ethanol peaks and then plateau for an extended period of time, perhaps exceeding 6 or more hours (Korsten et al. 1975). The plateau phenomenon probably represents the relatively steady level of production of AcH as the primary ethanol metabolizing systems become saturated (Freund and O'Hollaren 1965; Truitt 1971).

AcH does not appear to be evenly distributed in body tissues. The highest levels are in the liver, and it is possible that arterial blood may have higher concentrations than venous blood, and that these in turn are higher than cerebrospinal fluid (CSF), and that those exceed levels noted in the brain (Eriksson 1980*a*; Harada et al. 1978; von Wartburg 1979). The arterial–venous difference, however, may be an artifact of platelet properties that affect AcH levels directly after venipuncture (Eriksson 1980*a*).

NIH LIBRARY

Acetaldehyde (AcH) brain levels remain a subject of controversy. Early results indicated what appeared to be high concentrations in the brain after an ethanol load, some even demonstrating higher brain than venous levels (Truitt 1971; Westcott et al. 1980). These results probably represent the artifactual production of AcH in tissue samples and also differences between studies on the animal species used; the presence or absence of pretreatment with the ALDH inhibitor, disulfiram; the ethanol dose; and the time allowed for ethanol metabolism (Eriksson and Sippel 1977). More recent studies utilizing a substance to impair the artifactual production of AcH in brain tissue during analysis have demonstrated very low levels of AcH at even high alcohol doses (Eriksson and Sippel 1977; Sippel and Eriksson 1975; Tabakoff et al. 1976; Westcott et al. 1980).

Cerebrospinal fluid AcH levels are somewhat higher than those recorded in the brain; levels in the range of 15 μM to 40 μM in blood result in 5 μM to 20 μM levels in the interstitial fluid (Westcott et al. 1980). The differential between CSF and brain cell levels could reflect any of a variety of phenomena, including oxidation by ALDH in brain cells, destruction by aldehyde oxidase, oxidation within the capillaries by a low K_m ALDH, or destruction by aldehyde reductase, although this tends to have a low affinity for the short-chain aldehydes (Eriksson and Sippel 1977; Tabakoff and Gelpke 1975; Westcott et al. 1980). These findings do not preclude the possibility that specific brain areas might have even higher AcH levels, including the cerebellum or those areas in close proximity to the CSF (Lindros 1978; Truitt 1975).

Physiological Effects

Because of the potency of AcH, several important physiological reactions can occur. These reactions, of course, are dose dependent, with some of the more potent effects occurring only at mM levels and not at μM levels, including significant inhibition of protein synthesis and inhibition of various forms of ATPases (Perin et al. 1971; Tabakoff 1974).

One of the most important aspects of AcH action, probably occurring at lower blood levels, is stimulation of the release of the catecholamines (Eade 1959; Walsh 1971). Subsequently, changes in blood sugar (usually hyperglycemia) and decreases in potassium, along with increased levels of free fatty acids, can be observed (Truitt 1971; Walsh 1971).

AcH could also have important actions in the central nervous system (CNS). It has been hypothesized that some of the effects of ethanol, including the early stimulation that can be seen with that drug, could reflect the direct actions of AcH or the ratio between ethanol and AcH in the CNS (Truitt and Walsh 1971). It is also possible that AcH can produce levels of intoxication that mimic those observed with ethanol and can, itself, produce a lethal overdose (MacLeod 1950; Stotz et al. 1944; Truitt and Walsh 1971).

Although higher levels of AcH in peripheral blood may be associated with a lower level of ethanol intake in certain animal strains (Lindros 1978), direct application of AcH to the brain or cerebrospinal fluid can result in opposite reaction. Chronic administration of AcH to the cerebral ventricles, although not associated with many peripheral effects, does appear to be reinforcing (Brown et al. 1980; Myers and Veale 1969; Myers et al. 1980). Following AcH administration to the ventricle, animals have also demonstrated an increased preference for oral ethanol solutions (Brown et al. 1980).

In summary, AcH is a very potent substance that appears to be widely distributed in body fluids. Levels associated with ethanol intake can, at least theoretically, be associated with potent peripheral and CNS effects. Higher brain AcH levels might result in a greater ethanol preference in animals.

NIH LIBRARY

Possible Role of AcH in the Genesis of Alcoholism

Prior Studies

Over the past few years I have been interested in AcH as a possible risk factor associated with alcoholism. The possibility of such a role was documented when Freund and O'Hollaren (1965) demonstrated an almost twofold increased level AcH after the administration of 0.5 ml/lb of ethanol in an alcoholic with neurological damage when compared with physically well alcoholics and nonalcoholics. Subsequently, Truitt (1971) compared State hospital alcoholics who had been abstinent approximately 1 week with staff and students from a medical school. Both groups were administered between 0.5 and 0.75 gm/kg of ethanol (the equivalent of three to five drinks) as a 10 to 13 percent solution in grapefruit soda as two different doses given over 20 minutes. Using what was then a relatively new gas chromatograph (GC) technique for analysis of AcH levels, bloods were drawn every 30 minutes for the first 2 hours and then once per hour out to 5 hours. The investigator noted a trend for the alcoholics to demonstrate higher levels of AcH throughout the experiment, with a difference significantly higher (a threefold increase over controls) at 20 minutes after ingestion. These two studies raised the possibility that AcH might be higher in alcoholics than in controls, and that within alcoholics AcH may be associated with an increased level of physical pathology.

Korsten et al. (1975) compared six chronic alcoholics during their first 2 weeks of hospitalization with controls, making sure that all were in fairly good physical health without evidence of cirrhosis and that they had been off medication for a minimum of 72 hours. Administering intravenous ethanol as a 15 percent (v/v) solution in 5 percent dextrose to a level of 45 mm to 53 mm, the AcH levels were almost twice as high in alcoholics as in healthy controls—levels of 47 micromolar (μM) at peak blood levels versus 27 μM for controls, a significant difference. One subsequent report administering low alcohol doses (in the range of 10 to 100 milimolar (mM) of ethanol; 100 mg of ethanol per deciliter (mg/dl) equals approximately 22 mM) showed no AcH difference between alcoholics and controls (Lindros et al. 1979).

Taken together, these studies are generally consistent with a possible role of AcH either in the genesis of alcoholism or in some of the consequences of chronic high ethanol intake. However, it is difficult to draw definite conclusions because (a) most of the investigations did not control for usual alcohol intake patterns in the groups being compared; (b) most tested alcoholics after only a short period of abstinence; and, as will be discussed further, (c) the methodology employed in the preparation of AcH samples for analysis has been the subject of controversy. Some of these problems are addressed in the study of young men at high risk for future alcoholism described later in this paper.

If the AcH differences between alcoholics and controls are real, several interpretations are possible. First, the results could be the consequence of liver damage associated with ethanol intake because this damage could disturb the activity of the major enzyme metabolizing AcH, aldehyde dehydrogenase (ALDH) (Korsten et al.

1975). Higher levels of AcH in alcoholics could also reflect differences either in the way that they bind AcH to proteins or in the way that differential mechanisms remove this substance from the blood (von Wartburg 1979). It is also possible that higher AcH levels could result from a more rapid destruction of ethanol, perhaps through an enzyme system capable of induction, such as the microsomal ethanol oxidizing system (Korsten et al. 1975; von Wartburg 1979). An additional possibility rests not with the production of AcH in vivo but with differences between two groups on the level of artifactually produced AcH in vitro as bloods containing AcH and ethanol are being processed (von Wartburg 1979).

NIH LIBRARY

Data Supporting Genetics of Alcoholism

Regardless of the methodological problems, differences between alcoholics and controls could, regardless of the mechanism involved, represent a risk factor for the development of alcoholism. This speculation is interesting in light of the probability that alcoholism is a genetically influenced disorder. A genetic role in alcoholism is supported by the heavy familial nature of this problem, the higher level of concordance in monozygotic versus dizygotic twins, and the fourfold increased risk for alcoholism in the sons of alcoholics adopted out close to birth when compared with adopted-out sons of nonalcoholics (Colton 1979; Goodwin 1976; Schuckit in press). If alcoholism is genetically influenced, possible biological mediators of this tendency could involve differences between groups at high and low risk for the future development of alcoholism on the way that they metabolize ethanol, how they react to acute doses of the drug, differences in development of levels of tolerance, and a differential in the vulnerability to chronic consequences of repeated high levels of ethanol intake (Schuckit 1980). These are described briefly in table 1.

Research Paradigm and AcH Results

Because the investigations have compared established alcoholics with controls, none of the studies noted to this point help differentiate between elevated levels of AcH as a consequence of heavy ethanol intake versus as a factor increasing the risk for the future development of alcoholism. One way to test the possible role of AcH in the genesis of alcoholism is to look at "prealcoholics," individuals at high risk for the future development of alcoholism. Therefore, we carried out a study utilizing the nonalcoholic sons of alcoholics and compared their metabolism and acute reaction to ethanol to those of controls matched on demography, height/weight ratio, and quantity and frequency of alcohol intake.

Potential subjects were selected by distributing a questionnaire to a random sample of students and nonacademic staff at the University of Washington and the University of California, San Diego. This highly structured instrument allowed us to exclude those who fulfilled the criteria for alcoholism, drug abuse, or other major psychiatric disorders as defined by Goodwin and Guze (1979). For remaining subjects, those who reported alcoholism in a first degree relative were designated as the family history positive (FHP) or high-risk group and matched on demography, height/weight ratio, and drinking pattern with individuals with no such familial history—the family history negative (FHN), or low-risk group. Individuals were then brought to the laboratory at 7:00 a.m. after an overnight fast and administered 0.75 ml/kg of ethanol as a 20 percent solution in a room-temperature diet soft drink, which was consumed over 5 minutes.

Bloods for AcH analyses were drawn from an indwelling 18 gage "scalp-vein" needle inserted in an antecubital vein at least 30 minutes prior to drawing of the baseline (before ethanol) sample. AcH aliquots of 2 ml of blood were then taken at 15 minutes, 30 minutes, and every half hour after drinking over the 5-hour experiment.

For reasons discussed in detail later, our most recent procedure involves immediate mixing of venous blood with a fivefold dilution of ice cold PCA, cold centrifugation, incubation in a water bath, and "head space" sampling of the supernatant for gas chromatograph (GC) analysis. The GC parameters were oven temperature 150°C, injection temperature 145°C, FID temperature 155°C, and flow of carrier gas 32 ml/minute. The column is a Porapak 50 percent Q and 50 percent R by Supelco.

In the original analyses reported in 1978, the blood samples were handled somewhat differently. Blood was frozen and thawed with resulting hemolysis and artifactually elevated AcH levels (Eriksson 1980*b*, Schuckit and Rayses 1979). However, blood samples for FHP and FHN men were handled identically and, as shown in figure 1, the AcH levels were twofold higher for the FHP group.

These results, especially in the light of prior investigations, might be thought to adequately confirm the higher level of AcH in alcoholics by demonstrating that this level might represent a genetically influenced marker of an important difference in metabolism in individuals at high risk for developing alcoholism. However, methodological problems make such definite conclusions impossible. One purpose of this presentation is to further describe the methodological difficulties that have precluded a definite conclusion about the association between AcH and alcoholism and to share recent data gathered in FHP and FHN comparisons.

NIH LIBRARY

An Overview of the Methodology for Determining Acetaldehyde (AcH)

General Considerations

It is apparent that measures of AcH involve several compromises. First, most of AcH is produced and then metabolized directly in the liver, with the result that blood levels are only a rough approximation of what is actually going on within the body. Second, AcH is not evenly distributed in body areas, making tenuous any generalizations drawn from blood or breath AcH samples.

An additional problem is that most studies of AcH require that data gathered in vitro be compared with data demonstrated in vivo. This translation is not a safe assumption because it is probable that AcH in vivo might not exist in a totally free form (being reversibly bound to protein), while the same is not true of AcH added to blood samples with resulting different AcH levels generated from ethanol by blood samples in the two conditions (Stowell, Greenway, and Batt 1978). Additional problems include difficulties inherent in mixing exogenously added AcH with blood in a test tube and assuming that the dynamics of mixture and temperature would be the same as those noted in the body as well as difficulties that might be encountered by changes in blood samples noted only after venipuncture (Eriksson 1980*a*).

Similar problems occur when one attempts to generalize results from animal samples to those that might be noted in humans. To begin with, hemoglobin may have important properties in both the metabolism and binding of blood AcH, and this substance contains four active sulfhydryl groups in the rat but only two in man

(Eriksson et al. 1977*b*)—properties that tend to make rat blood metabolism of AcH different from that noted for humans. Related to this, the artifactual production of AcH from ethanol in rat blood samples is quite different from that in humans, and there might be equally important differences in the rate of destruction of AcH (Kinoshita 1974; Lindros 1978; Westcott et al. 1980).

Additional important problems are inherent in the manner in which samples are prepared for AcH determinations. As shown in tables 2 and 3, these include problems with destruction of AcH within samples and the artifactual production of AcH from ethanol within samples.

Loss of AcH Drug Sample Preparation

Some immediate loss of AcH occurs following venipuncture. This rapid reaction takes place over 15 to 30 seconds, during which the loss may be as much as 50 percent (Eriksson 1980*a*). This reaction may be related to a binding reaction of AcH secondary to platelet activation and in turn may be responsible for the erroneous reports of arterial–venous differentials in AcH levels. These difficulties are at least partially circumvented by immediately deproteinizing the samples, working with heparinized plasma samples rather than whole blood, and possibly by using an indwelling venous catheter, delaying blood withdrawal until perhaps 30 minutes after venipuncture. It should be noted that not all investigators have observed the same magnitude of rapid loss following the insertion of a needle (Stowell 1979).

An additional loss of AcH can occur if blood samples containing ethanol are allowed to stand at room temperature. A similar reaction is not observed when blood is kept cold (4°C), but is seen with ethanol incubated in saline with red blood cells (RBCs) added (von Wartburg 1979). This reaction is apparently primarily due to the actions of blood proteins and ALDH present in red cells (Eriksson 1980*a*) and should be reversed or retarded by keeping bloods cold (Lindros 1978; Stowell, Greenway, and Batt 1978; Stowell, Crow, Greenway, and Batt 1978) and by deproteinization of blood samples (Deitrich 1966; Lindros 1978; von Wartburg 1979). No matter what the mechanism, the major loss appears to involve the conversion of AcH to acetate (Stowell, Greenway, and Batt 1978). If so, then in addition to removing RBCs and blood proteins, further retardation in the reaction could occur by adding an ALDH inhibitor such as chloralhydrate (von Wartburg 1979).

Another potential loss of AcH, through evaporation to the atmosphere, is reported to be quite small (Stowell, Greenway, and Batt 1978) and can be minimized by keeping samples cold and stoppered. However, some of our data questions the magnitude of loss. Considering the volatile nature of AcH, transferring biological solutions from container to container could, at least theoretically, result in some significant loss to the atmosphere. To test this hypothesis we developed a "closed system" tested on 10 subjects whereby 1 ml of blood from the subject was injected into a closed flask through a rubber septum. The flask (kept on ice) already contained a fivefold dilution of PCA as well as 20 mM thiourea. The subsequent solution, including blood, was then mixed and incubated in a water bath at 65°C and the head-space air sampled. As shown in figure 2, the results are somewhat higher for this closed system when compared with our usual "open system" method of analysis described earlier.

An important step in decreasing the artifactual loss of AcH from biological samples involves separating the serum or plasma ethanol from RBCs and protein that could destroy the AcH. The usual approach here is done with a PCA solution kept on ice and must be carried out with a dilution of one part blood to a minimum of five parts and preferably nine parts PCA (Stowell, Greenway, and Batt 1978). The reasons for the high PCA dilution relate to spurious production of AcH, as described in the next section.

One effort to decrease the AcH production during deproteinization has been to add thiourea, because this substance might interfere with an ascorbic acid free radical mechanism of ethanol oxidation, as discussed below. However, if concentrations of this substance in excess of 20 mM are used, this approach might artifactually lower the amount of reactive AcH in the solution (Eriksson 1980*a*). Finally, because ADH can also take AcH to ethanol, in vitro analyses of AcH in the absence of ethanol can result in seriously low AcH determinations (Lindros 1978).

Spurious AcH Production

The discussion to this point has foreshadowed the next major difficulty in preparing biological samples for AcH determinations,

NIH LIBRARY

the artifactual production of AcH. As noted in table 3, one problem can occur in vivo when ethanol is given as bourbon because some such beverages contain levels of AcH themselves (Truitt and Walsh 1971), making it important that analyses be carried out after the administration of laboratory ethanol.

This problem is minor compared with the rise in AcH noted during the deproteinization process with PCA. The magnitude of this reaction increases when samples are heated (Eriksson 1980*a*; Eriksson et al. 1975, 1977*a*), when less dilution with PCA is done (Stowell, Greenway, and Batt 1978), and when ethanol concentrations are higher (Stowell et al. 1977). The reason for the decreased production of AcH with an increasing dilution with PCA is unknown, but could be related to the actual speed of deproteinization (Stowell et al. 1977) or the rapidity of solution cooling. It is also important to note that there is almost no AcH produced in the protein-free supernatants (Stowell et al. 1977). As part of our experiment, bloods from 10 FHP-FHN pairs were processed using a twofold PCA dilution. The resulting AcH levels uniformly peaked higher than 5 µg/ml with little difference between groups. Clearly, use of too little PCA results in unacceptably high spurious AcH production.

Several theories might help explain the source of AcH associated with deproteinization. First is the possible release of AcH during protein precipitation as a source of spuriously high AcH levels during preparation of blood samples. Several factors favor possible AcH binding to blood constituents, including the observation that exogenously added AcH disappears rapidly in blood samples whereas endogenously produced AcH does not (Stowell, Greenway, and Batt 1978) and the documentation of AcH binding in rat blood (Eriksson 1980*a*; Eriksson, Sippel, and Forsander 1977*a*). In both human and animal samples, an increasing amount of AcH is liberated if the precipitation with PCA is carried out at 25°C rather than at 4°C (Eriksson 1980*a*). It is thus possible that the usual PCA precipitation methods result in determination of both AcH that had been "free" in the blood and that released from RBCs and proteins (Pikkarainen et al. 1979). The fact that actual AcH binding in human blood had not been demonstrated to be as relevant as it is in rat samples relegates this explanation to a possible but unproven position in human work.

Second, the nonenzymatic (so-called because no enzymatic mechanism is known) production of AcH from ethanol during deproteinization could be related to the denaturation of oxyhemoglobin to methemoglobin (MacLeod 1950). This produces a superoxide radical (O_2^-), which could itself mediate ethanol oxidation (Stowell et al. 1977). However, it is important to note that the addition of ethanol to a superoxide solution produces no AcH in vitro (Nishikimi et al. 1972).

Another important nonenzymatic production of AcH from ethanol appears to be related to a free radical oxidation of ethanol mediated by ascorbic acid (Sippel 1973). This reaction can be stopped by the addition of thiourea. The fact that thiourea is effective in solutions from tissue samples (e.g., brain) but not effective in blood samples indicates that two different mechanisms are involved (Eriksson 1980*a*; Lindros 1978; von Wartburg 1979). The final result is that thiourea is probably ineffective in controlling spurious production of AcH in human blood samples.

The magnitude of the spurious production of AcH during deproteinization is not known. Using a ninefold PCA dilution, 30 mM ethanol in blood was associated with AcH production of between 5 μM and 7 μM in vitro, with 10 μM AcH seen with blood ethanol concentrations as high as 40 mM (Korsten et al. 1975; Stowell et al. 1977). Once precipitation has been completed, after centrifugation the supernatant can be safely stored at 4°C for 24 hours or more with no more artifactual AcH production (Stowell et al. 1977).

In summary, the major difficulties with production of artifactual AcH appear to occur during precipitation of the blood proteins with PCA (a step taken at least in part to avoid artifactual destruction of AcH in the first place). The problem can be minimized by using a fivefold to ninefold dilution of PCA, keeping samples cold, and for tissue but not blood samples, utilizing approximately 20 mM solutions of thiourea. The levels of AcH produced are modest but could, at least theoretically, spuriously raise estimates in body fluids. Figure 3 demonstrates results of AcH analyses on 5 FHN-FHP pairs (10 individuals) using these procedures coupled with the GC parameters reported earlier. The twofold higher AcH for FHP men remains.

Several additional solutions to the problems of spurious production and destruction of AcH have been advocated. One is through production of a correction factor whereby the addition of various concentrations of ethanol to blood samples in vitro followed by PCA deproteinization and AcH measurement could be used as models for the amount of AcH produced (Eriksson 1980*a*; Stowell, Crow, Greenway, and Batt 1978; Stowell, Greenway, and Batt 1978).

NIH LIBRARY

There are numerous difficulties inherent in such a correction factor, including the use of blood from blood banks that has been pretreated in various ways, difficulties in adequately mixing the ethanol with the blood and incubating it in a manner similar to that which could be expected in in vivo conditions, and the apparent faster disappearance of AcH in in vitro samples than in in vivo bloods. In fact, in our hands, utilizing the correction factor, higher levels of AcH appear to be generated than are seen at comparable blood ethanol concentrations, with the result that the final corrected AcH levels can be less than zero (von Wartburg 1980). The correction curve generated from individual to individual appears to vary greatly, making such a procedure potentially unreliable (Pikkarainen et al. 1979).

The Plasma Method

Another mechanism used to try to avoid the spurious production and destruction of AcH involves utilization of heparinized plasma samples rather than whole blood. The rationale is based on the observation of decreased destruction of AcH in blood samples where RBCs are rapidly separated from the rest of the blood constituents (Pikkarainen et al. 1979; von Wartburg 1979; von Wartburg and Ris 1979). A second advantage to this procedure is that, at least theoretically, it would avoid the spurious nonenzymatic production of AcH during the precipitation of blood proteins, a theory backed up by the observation of little spurious production of AcH in heparinized samples (Stowell, Greenway, and Batt 1978). The amount of AcH produced under such circumstances in plasma samples with 20 mM ethanol is less than 1 μM of AcH (Stowell, Greenway, and Batt 1978). In carrying out such experiments, it appears as if the specific anticoagulant, EDTA or heparin, doesn't matter (Stowell, Greenway, and Batt 1978).

To adequately control for the potential disappearance of AcH, some investigators believe that the samples should be treated with an aldehyde dehydrogenase inhibitor, the most effective and useful appearing to be chloral hydrate (von Wartburg and Ris 1979). In this method, blood containing ethanol is immediately mixed with heparin and 50 mM chloral hydrate and then centrifuged to separate out RBCs. The solution is then deproteinized with 0.1 ml 60 percent PCA and placed in a stoppered container for analysis, because it appears that proteins can be safely precipitated without spurious production of AcH after the RBCs have been separated out (von Wartburg 1979). Deletion of PCA precipitation results in approximately a 30 percent loss of AcH, probably secondary to irreversible binding of AcH with plasma protein, but it appears that the presence of ethanol with chloral hydrate does not change the results (von Wartburg 1979).

To gather more information on the question of comparability between blood and plasma results, we carried out two experiments. In the first, as demonstrated in table 4, blood was sampled from an indwelling venous catheter, using a heparin lock that had been inserted at least half an hour before sampling procedures were carried out. Two ml of blood were then added to enough ethanol to make a 50 mg/dl solution; after 5 seconds either 1, 2, 4, or 8 μg/ml of AcH were added, the bloods then allowed to stand at room temperature for 20 seconds (the approximate amount of time it might take to actually draw blood), and the samples processed by two different methods. In the first, our usual system of fivefold dilution PCA, the addition of thiourea, and centrifugation (all under cold conditions) was carried out and samples prepared for GC analysis. In the second condition, the blood was added to 2 ml of a 50 mM solution of chloral hydrate, centrifuged in the cold at 2,500 rpm for 15 minutes, 2 ml of supernatant then removed and precipitated with 0.1 ml of 60 percent PCA, centrifuged, and bloods prepared for GC analysis. It can be seen that after correction for the different levels of dilution used in the two procedures, the resulting AcH values were relatively equivalent. All determinations for both methods were carried out in triplicate.

Next we tested the comparability of blood and plasma results in vivo. Table 5 gives preliminary data on three individuals who were administered the 0.75 ml/kg of ethanol as part of our regular procedure. Analysis of these AcH samples generated in vivo once again demonstrated the rough comparability between the plasma and whole blood methods. However, there was a relatively large amount of variation within the triplicate analyses of the plasma samples, raising questions about the reliability of this plasma procedure in vivo.

Another possible method for circumventing the procedural problems involved in preparation of AcH samples has been proposed by Stowell (1979), in which blood is added to a semicarbazide heparin solution to stabilize the AcH so that none is lost during heparinization and subsequent centrifugation of the RBCs. As a result, low volumes of PCA can be used, which serves the dual

NIH LIBRARY

purpose of deproteinizing and releasing the AcH from the semicarbazide binding. This modification of the heparin or plasma approach results in good recovery of in vitro added AcH and gives results comparable to the PCA method while avoiding the necessity of using the correction factor and obviating the problems of decreased sensitivity that can be noted with high levels of PCA dilution (Stowell 1979). Because of the possible lowered reliability of the chloral hydrate nonsemicarbozide approach, we plan a series of experiments comparing the blood to semicarbozide plasma methods.

Breath Samples

One final variant must be mentioned. As briefly noted earlier, a small amount of AcH is excreted directly in the breath. Measurement of breath AcH would take advantage of the fact that the hepatic blood goes almost directly to the lungs and thus this methodology might better gage the actual level of AcH in hepatic blood (Eriksson 1980*a*; Freund and O'Hollaren 1965). The method has the additional benefits of allowing measurement of AcH without manipulating samples by precipitation (Lindros 1978; Eriksson 1980*a*) and ease of serial sampling. Determinations of breath AcH appear to be proportional to blood ethanol, and repeated results on one person over 10 sessions appear to be quite similar (Lindros 1978; Freund and O'Hollaren 1965). Utilizing gas chromatographic (GC) techniques, breath AcH levels appear to be in the range of 500 μg/100 ml to 900 μg/100 ml at peak blood ethanol levels after a modest dose, with results often demonstrating a plateau above 15 μg/100 ml to 25 μg/100 ml of ethanol (Freund and O'Hollaren 1965).

As might be expected from the discussion of blood AcH levels, the results are not as clearcut as they might first appear. The major difficulty occurs because the lungs might change the AcH levels, an action at least in part related to the complicated behavior of AcH in vapor-liquid interface in dilute solutions (Lindros 1978). In addition, ethanol can be oxidized within the lungs by microbes or lung microsomes or by lung ADH (Eriksson 1980*a*; Pikkarainen et al. 1979; von Wartburg 1979). The result is that it is difficult to draw conclusions about the relationship between blood and breath AcH. Several investigations to date have indicated a lack of comparability of findings (Eriksson 1980*a*; Freund and O'Hollaren 1965).

AcH Measurement

It is apparent that the major problems in determining AcH rest with the preparation of samples. Once samples are prepared, the amount of AcH in a prepared biological sample can be determined by one of several methods.

Until recent years, most analyses used colorimetric, spectrophotometric, radiochemical, or enzymatic assays. With the possible exception of enzymatic assays (Stowell et al. 1978), these methodologies lack specificity and sensitivity for AcH (von Wartburg 1979).

The enzymatic assay has been improved by the development of a purified form of ALDH (Eriksson 1980*a*; Stowell, Crow, Greenway, and Batt 1978). The procedure utilizing sheep liver ALDH and fluorimetrically measuring the amount of NADH produced can be automated, and results are equivalent to those related by the GC as described here.

The most widely used procedure now involves the highly sensitive and specific method on the gas chromatograph (GC). A modification of usual GC procedures had to be developed because injection of tissue samples or blood directly in the column results in the deposition of nonvolatile and slowly decomposing organic compounds that then interfere with the AcH and ethanol analyses (Lindros 1978). Therefore, the head-space technique was developed whereby solutions containing AcH (usually the supernatants of samples processed as discussed above) are placed in a stoppered bottle and heated to a constant temperature over a fixed period of time. Then a given amount of the air in the "head space" above the liquid in the closed container is injected into the GC for analysis (Duritz and Truitt 1966; Schuckit and Rayses 1979). Using columns developed for AcH and ethanol analyses, this procedure is highly specific and sensitive (Lindros 1978; Stowell, Crow, Greenway, and Batt 1978), but some contamination can occur through improper sampling of air, deterioration of rubber stoppers, and some irreversible binding to the column packing material (Eriksson 1980*a*). For our analyses, we use a Poropak glass column (50 percent Q, 50 percent R) supplied by Supelco. The GC parameters were injection temperature 145°C, FID temperature 155°C, and carrier gas flow rate 32 ml/min. One further, and as yet relatively untested, modification of the procedure involves use of a paper disk with 10 μl to 50 μl of blood placed in a closed bottle with sampling of the head-space air (Coldwell et al. 1971).

Some Conclusions

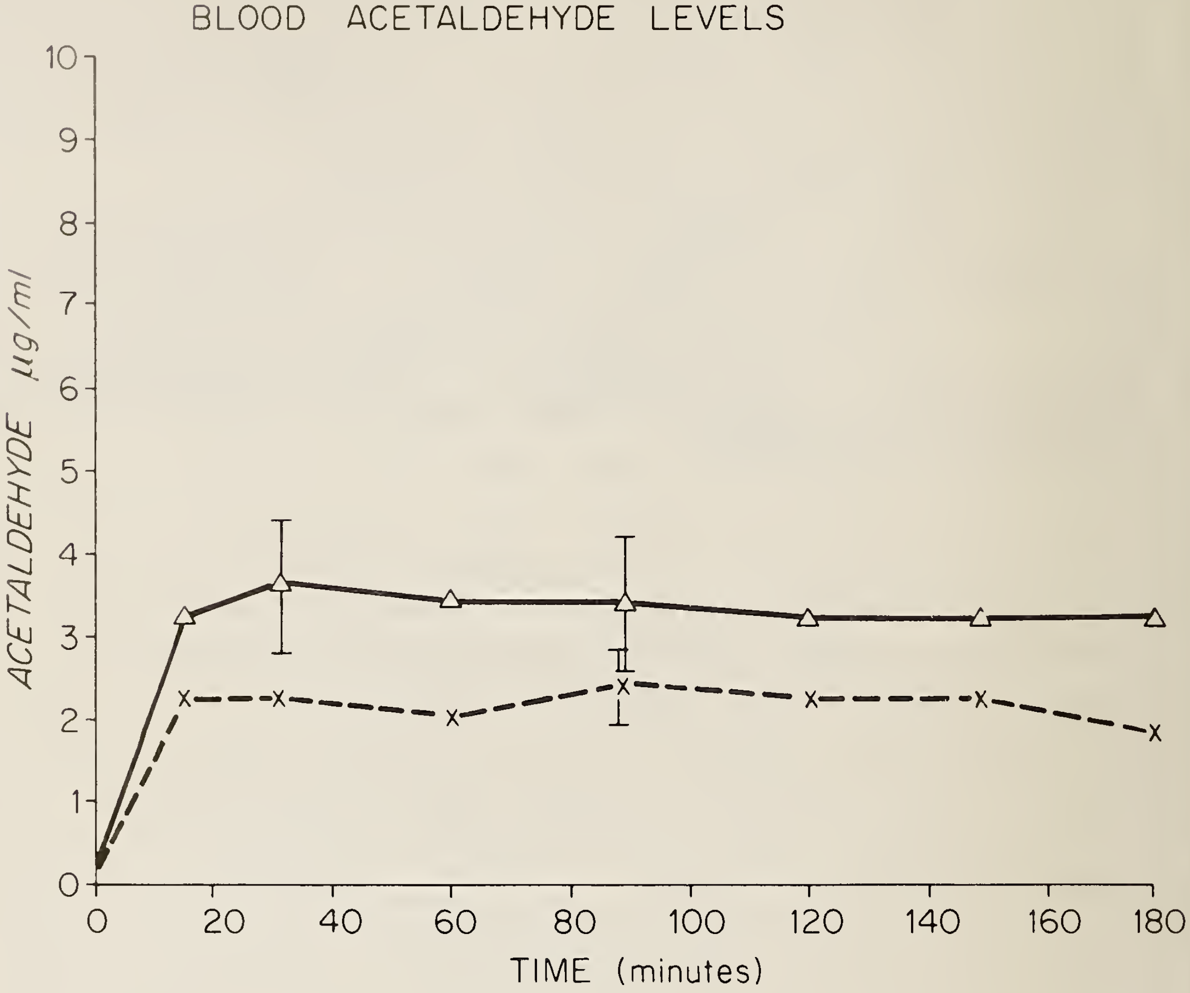
Acetaldehyde is a highly volatile, biologically reactive compound that, at least theoretically, is widely distributed in body fluids and tissues. This substance has been the focus of much interest because it might help explain some of the physiological actions of ethanol, a factor of great importance, considering the level of ignorance regarding the mechanisms of action of this widely used drug. An additional reason for interest has been the demonstration of higher levels of AcH in alcoholics and their relatives, pointing to this interesting substance as a possible mediator of organ damage in alcoholics and/or a propensity toward alcoholism itself.

One stumbling block in our understanding of AcH has been disagreement about the proper methodology for processing biological samples containing AcH. These have been greatly complicated by differences between in vitro and in vivo experiments and difficulties in translating between animal and human research. The major problems involve the destruction of AcH within samples by protein binding, and metabolism by ALDH in RBCs, as described in table 2. The second major difficulty involves the spurious production of AcH, which can occur through ADH present in sample solutions and through release of bound AcH or by a nonenzymatic production of AcH that occurs during protein precipitation, as noted in table 3. Although the latter problem can be corrected in supernatant solutions over tissue samples (e.g., liver or brain) by thiourea, no perfect answer has been developed for handling samples of human blood. Most of these difficulties can be minimized by using fivefold to ninefold dilutions of PCA, by keeping samples cold, and by separating out proteins and RBCs as rapidly as possible. The use of a correction curve generated from in vitro experiments should be considered, but may yield results below zero, another difficulty inherent in translating from in vitro to in vivo results. It is, therefore, apparent that the present methodologies for handling AcH in biological samples are far from perfect, making it difficult to compare results from different laboratories and imperative that investigators present thorough descriptions of their methods.

The possible differences in AcH levels after ethanol intake for young men with family histories of alcoholism and controls remains an area of active interest in our laboratory. Utilizing two very different methodologies for handling blood samples, we have twice demonstrated a twofold higher level of AcH in the children of alcoholics when compared with controls matched on demography, height/weight ratio, and drinking history. One experiment, using methods that generated very high spurious levels of AcH, showed no differences between groups. Considering the identical handling of samples for FHP and FHN men, it is probable that some real difference in AcH exists, although the source of the differential, whether in the liver or in the blood, is unknown.

Future sets of experiments will use ninefold dilutions with PCA and delete the use of thiourea. In addition, we hope to carry out a full set of experiments utilizing both breath determinations and blood analyses using the plasma methodology suggested by von Wartburg (1979) or the semicarbazide method of Stowell (1979) and Pikkarainen et al. (1979).

Figure 1. **Blood Acetaldehyde Levels in μg/ml for 20 Nonalcoholic Young Men With Alcoholic Relatives and Controls After Ingestion of 0.75 ml of Ethanol/kg**



SOURCE: Schuckit and Rayses. *Science*, 203:54-55, 1979. Copyright 1979 by the American Association for the Advancement of Science.

Figure 2. **Pilot Data on Acetaldehyde Levels in μg/ml for the Usual "Open" System (open circles) and the Same Samples Processed in Enclosed Flasks, the "Closed" System (closed circles), Demonstrating the Loss of Acetaldehyde to the Atmosphere When Tubes Are Open During Sample Processing**

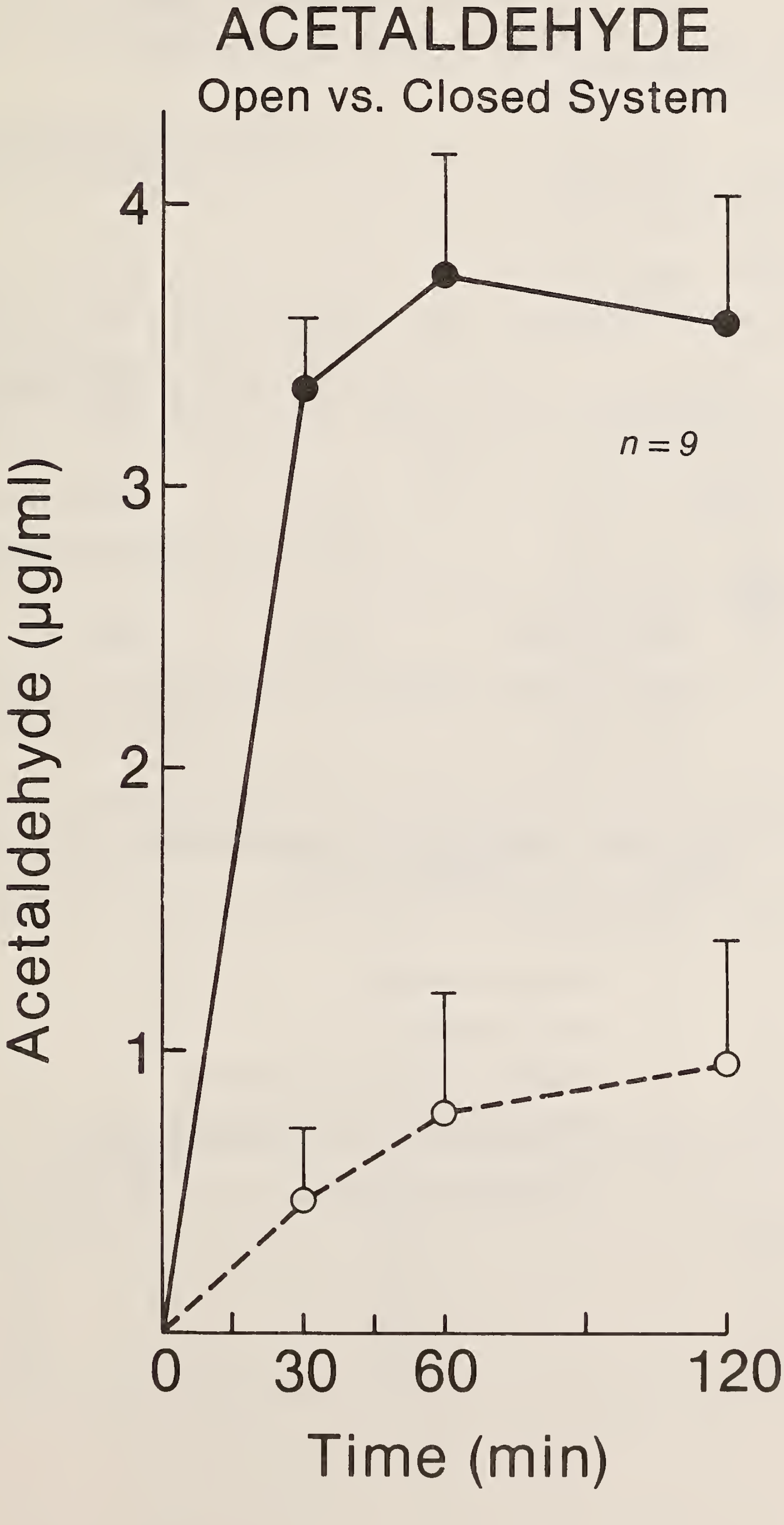


Figure 3. **Acetaldehyde Levels After 0.75 ml/kg of Ethanol for Five Family History Positive/Family History Negative Matched Pairs Utilizing a Fivefold PCA Dilution**

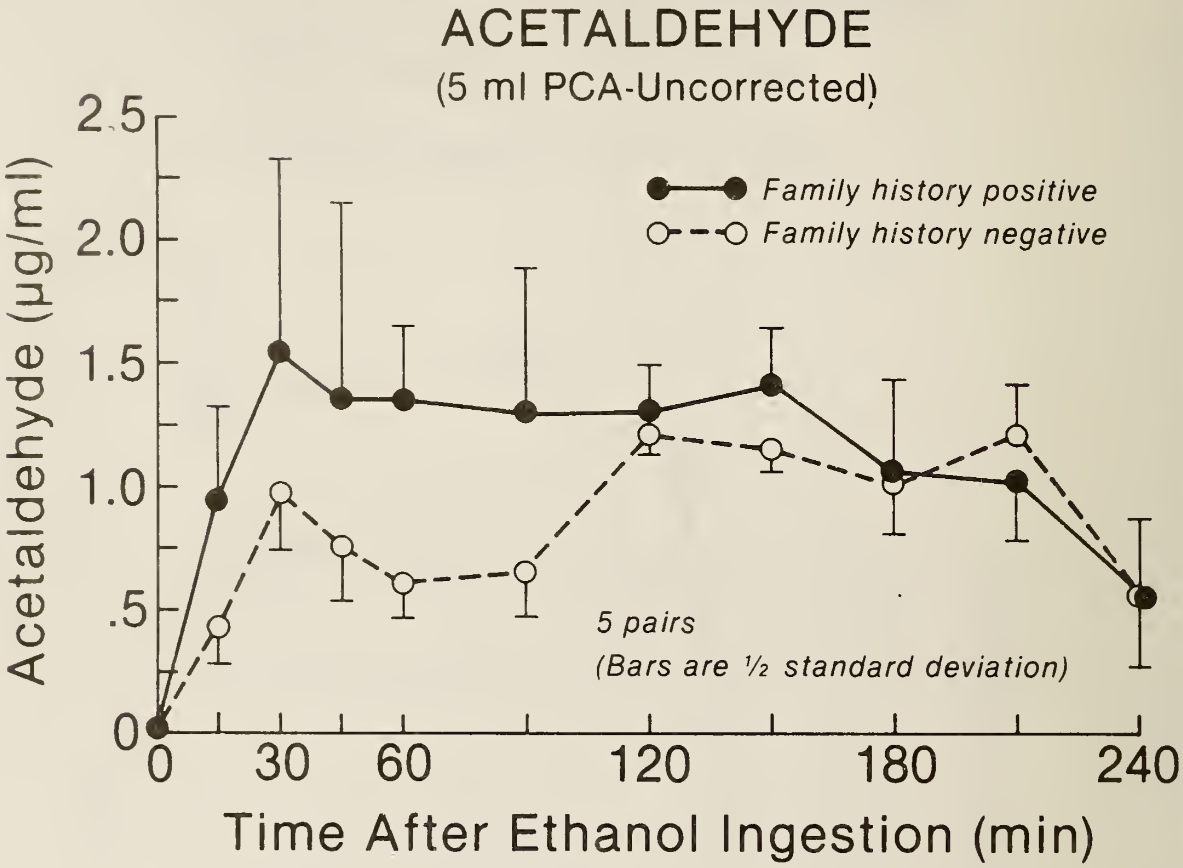


Table 1. **Possible Biological Mechanisms of a Genetic Propensity in Alcoholism**

Differences in —

1. Ethanol metabolism
2. Acute reaction
3. Subacute reaction (e.g., tolerance)
4. Vulnerability to chronic consequences
5. Personality attributes

Table 2. Destruction of Acetaldehyde

Problem	Possible Solution
Rapid loss after venipuncture	Deproteinize immediately Heparinize Use indwelling catheter
Slower loss in presence of RBCs and protein	Keep cold Heparinize and centrifuge Deproteinize Use ALDH inhibitor
Loss to atmosphere	Keep cold Use stopper bottles Use "closed" system
Thiourea (> 20 mM)	Use none or dilute solutions
Conversion to ethanol by ADH in vitro	Analyze with ethanol

Table 3. Spurious Production of Acetaldehyde

Problem	Possible Solution
Present in beverage	Use pure ethanol
Nonenzymatic production	Keep cold 5X–9X PCA dilution Thiourea (for tissues only) Plasma Correction curve
Release from bound form	Deproteinize Keep cold

Table 4. AcH Values in Blood and Plasma (In Vitro)

Amt. AcH Added (μg/ml)	$\bar{X}$ AcH – Blood (μg/ml)	$\bar{X}$ AcH – Plasma (μg/ml)
1	1.424	1.094
2	1.985	1.938
4	3.518	3.812
8	6.494	6.892

Table 5. Comparison of Blood and Plasma AcH (μg/ml) In Vivo

Time (min)	Blood	Plasma
30	0.350	0.157
60	0.825	0.397
90	3.446	2.033

NIH LIBRARY

References

Brown, W.B.; Amit, Z.; and Smith, B. Intraventricular self-administration of acetaldehyde and voluntary consumption of ethanol of rats. *Behav Neural Biol*, 28:150-155, 1980.

Coldwell, B.B.; Solomonraj, G.; Trenholm, H.L.; and Wiberg, G.S. The gas chromatographic estimation of ethanol, acetaldehyde, and acetone in ethanol metabolism studies. *Clin Toxicol*, 4:99-113, 1971.

Colton, N.S. The familial incidence of alcoholism. *J Stud Alcohol*, 40:89-116, 1979.

Deitrich, R.A. Tissue and subcellular distribution of mammalian aldehyde-oxidizing capacity. *Biochem Pharmacol*, 15:1911-1921, 1966.

Duritz, G., and Truitt, E.B. Importance of acetaldehyde in the action of ethanol on brain norepinephrine and 5-hydroxy-tryptamine. *Biochem Pharmacol*, 15:711-721, 1966.

Eade, N.R. Mechanism of sympathominetic action of aldehydes. *J Pharmacol Exp Ther*, 127:29-34, 1959.

Eriksson, C.J.P. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation-II. *Biochem Pharmacol*, 26:249-252, 1977*a*.

Eriksson, C.J.P. "Regulation of the Hepatic Acetaldehyde Metabolism During Ethanol Oxidation in Rats." Dissertation. Helsinki, 1977*b*.

Eriksson, C.J.P. Problems and pitfalls in acetaldehyde determinations. *Alcohol Clin Exp Res*, 4:22-29, 1980*a*.

Eriksson, C.J.P. Elevated blood acetaldehyde levels in alcoholics and their relatives: A reevaluation. *Science*, 207:1383-1384, 1980*b*.

Eriksson, C.J.P., and Sippel, H.W. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation-I. *Biochem Pharmacol*, 26:241-247, 1977.

Eriksson, C.J.P.; Sippel, H.W.; and Forsander, O.A. The role of acetaldehyde in the actions of ethanol. *Finn Foundat Alcohol Stud* (Helsinki), 23:9-11, 1975.

Eriksson, C.J.P.; Sippel, H.W.; and Forsander, O.A. The determination of acetaldehyde in biological samples by head-space gas chromatography. *Anal Biochem*, 80:116-124, 1977*a*.

Eriksson, C.J.P.; Sippel, H.W.; and Forsander, O.A. The occurrence of acetaldehyde binding in rat blood but not in human blood. *Fed Eur Biochem Soc*, 75:205-208, 1977*b*.

Freund, G., and O'Hollaren, P. Acetaldehyde concentrations in alveolar air following a standard dose of ethanol in man. *J Lipid Res*, 6:471-477, 1965.

Goodwin, D. *Is Alcoholism Hereditary*? New York: Oxford University Press, 1976.

Goodwin, D.W., and Guze, S.B. *Psychiatric Diagnosis*. New York: Oxford University Press, 1979.

Harada, S.; Agarwal, D.P.; and Goegge, H.W. Isozyme variations in acetaldehyde dehydrogenase (E.C. 1.2.1.3.) in human tissues. *Hum Genet*, 44:181-185, 1978.

Horton, A.D., and Guerin, M.R. Determination of acetaldehydes and acrolein in the gas phase of cigarette smoke using cryothermal gas chromatography. *Tobacco*, 176:45-47, 1974.

Kinoshita, M. Gas chromatographic determination of blood acetaldehyde levels after ingestion of ethanol in man. *Jpn J Stud Alcohol*, 9:1-8, 1974.

Korsten, M.A.; Matsuzaki, S.; Feinman, L.; and Lieber, C.S. High blood acetaldehyde levels after ethanol administration. *N Engl J Med*, 292:386-389, 1975.

Li, T.-K.; Bosron, W.F.; Dafeldecker, W.P.; Lange, L.G.; and Vallee, B.L. Isolation of

II-alcohol dehydrogenase of human liver: Is it a determinant of alcoholism? *Proc Natl Acad Sci USA*, 74:4378-4381, 1977.

Lieber, C.S. Pathogenesis and early diagnosis of alcoholic liver injury. *N Engl J Med*, 298:888-893, 1978.

Lindros, K.O. Acetaldehyde—Its metabolism and role in the actions of alcohol. *Res Adv Alcohol Drug Prob*, 4:111-175, 1978.

Lindros, K.O.; Salaspuro, M.; and Pikkarainen, P. Studies on the role of the ADH pathway in increased ethanol elimination after chronic alcohol intake in the rat and man. *Alcohol Aldehyde Metabol Systems*, 3:210-217, 1979.

MacLeod, L.D. Acetaldehyde in relation to intoxication by ethyl alcohol. *Q J Stud Alcohol*, 11:385-390, 1950.

Majchrowicz, E., and Mendelson, J.H. Blood concentrations of acetaldehyde and ethanol in chronic alcoholics. *Science*, 168:1100-1102, 1970.

Myers, R.D., and Veale, W.L. Alterations in volitional alcohol intake produced in rats by chronic intraventricular infusions of acetaldehyde, paraldehyde or methanol. *Arch Int Pharmacodyn Ther*, 180:100-113, 1969.

Myers, R.D.; Melchior, C.; and Swartzwelder, H.S. Amine-aldehyde metabolites and alcoholism: Fact, myth or uncertainty. *Substance Alcohol Actions/Misuse*, 1:223-228, 1980.

Nishikimi, M.; Rao, N.A.; and Yagi, K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. *Biochem Biophys Res Commun*, 46:849-852, 1972.

Perin, A.; Scalabrini, G.; Sessa, A.; and Arnaboldi, A. In vitro inhibition of protein synthesis in rat liver as a consequence of ethanol metabolism. *Biochim Biophys Acta*, 366:101-103, 1971.

Pikkarainen, P.H.; Salaspuro, M.P.; and Lieber, C.S. A method for the determination of "free" acetaldehyde in plasma. *Alcohol Clin Exp Res*, 3:259-261, 1979.

Ridge, J.W. The metabolism of acetaldehyde by the brain in vivo. *Biochem J*, 88:95-100, 1963.

Schuckit, M.A. Alcoholism and genetics: Possible biological mediators. *Biol Psychiatry*, 15:437-447, 1980.

Schuckit, M.A. Twin studies on substance abuse: An overview. *Acta Med Gemellol*, in press.

Schuckit, M.A., and Rayses, V. Ethanol ingestion: Differences in blood acetaldehyde concentrations in relatives of alcoholics and controls. *Science*, 203:54-55, 1979.

Sippel, H.W. Non-enzymatic ethanol oxidation in biological extracts. *Acta Chem Scand*, 27:541-550, 1973.

Sippel, H.W., and Eriksson, C.J.P. The acetaldehyde content in rat brain during ethanol oxidation. *Finn Foundat Alcohol Stud* (Helsinki), 23:149-157, 1975.

Stotz, E.; Westerfeld, W.W.; and Berg, R.L. Metabolism of acetaldehyde with acetoin formation. *J Biol Chem*, 152:41-50, 1944.

Stowell, A.R. An improved method for the determination of acetaldehyde in human blood with minimal ethanol interference. *Clin Chim Acta*, 98:201-205, 1979.

Stowell, A.R.; Greenway, R.M.; and Batt, R.D. Acetaldehyde formation during deproteinization of human blood samples containing ethanol. *Biochem Med*, 18:392-401, 1977.

Stowell, A.R.; Greenway, R.M.; and Batt, R.D. Stability of acetaldehyde in human blood samples. *Biochem Med*, 20:167-179, 1978.

Stowell, A.R.; Crow, K.E.; Greenway, R.M.; and Batt, R.D. Determination of acetaldehyde in blood using automated distillation and fluorometry. *Anal Biochem*, 84:384-392, 1978.

Tabakoff, B. Inhibition of sodium, potassium, and magnesium activated ATPases by acetaldehyde and "biogenic" aldehydes. *Res Commun Chem Pathol Pharmacol*, 7:621-624, 1974.

Tabakoff, B., and Gelpke, C.C. Alcohol and aldehyde metabolism in brain, in biochemical pharmacology of ethanol. *Adv Exp Med Biol*, 56:141-142, 1975.

Tabakoff, B.; Anderson, R.A.; and Ritzman, R.F. Brain acetaldehyde after ethanol administration. *Biochem Pharmacol*, 25:1305-1307, 176.

Truitt, E.B. Blood acetaldehyde levels after alcohol consumption by alcoholic and nonalcoholic subjects. *Biol Aspects Alcohol*, 3:212-232, 1971.

Truitt, E.B., and Walsh, M.J. The role of acetaldehyde in the actions of ethanol. *Biol Alcohol*, 1:161-195, 1971.

von Wartburg, J.P. "Acetaldehyde." Paper presented at the meeting of the British Association for Psychopharmacology, London, 1979.

von Wartburg, J.P. The acetaldehyde correction curve. Personal communication, November 1980.

von Wartburg, J.P., and Ris, M.M. Determination of acetaldehyde in human blood. *Experientia*, 35:1682-1683, 1979.

Walsh, M.J. Role of acetaldehyde in the interactions of ethanol with neuroamines. *Biol Aspects Alcohol*, 3:232-254, 1971.

Westcott, J.Y.; Weiner, H.; Shultz, J.; and Myers, R.D. In vivo acetaldehyde in the brain of the rat treated with ethanol. *Biochem Pharmacol*, 29:411-417, 1980.

Breath Acetaldehyde: Highly Sensitive Evaluation of Endogenous and Exogenous Ethanol Metabolism*

John R. Dannecker, Jr., Edward G. Shaskan, and Roger E. Meyer

Introduction

Acetaldehyde (AcH) is the first metabolic product of ethanol in the human body and is a constituent of many alcoholic beverages. It has been reported that AcH is 10 to 30 times more toxic than ethanol itself (Kricka and Ciark 1979). Abnormalities in the metabolism of ethanol that give rise to varying concentrations of AcH in blood have been implicated in etiologic and pathogenic aspects of alcoholism (Cederbaum et al. 1974). Supporting this implication are findings from numerous laboratories, including those of Korsten et al. (1975), who reported that upon administering an equivalent dose of ethanol, alcoholics had a higher resultant mean blood AcH level than did matched controls; and Schuckit and Rayses (1979), who reported that mean blood AcH level was greater after ethanol administration in first degree relatives of alcoholics compared with age-matched controls with no familial alcoholism.

However, research into the metabolism of ethanol has been fraught with conflicting results, largely because of technical difficulties in measuring AcH in blood and breath accurately and

---

Note: Figures and table appear at end of paper.

*This research was supported by NIAAA grant 1 P50 AA 03510-03, Center for the Study of Alcoholism.

reproducibly. This problem is due to formation and disappearance reactions in blood even after the specimen has been collected in vitro (Eriksson 1980) and to small breath sample size leading to poor reproducibility and little sensitivity. Our experience with the perchloric acid method (Eriksson et al. 1977) for blood AcH determinations confirmed the accepted necessity of a correction curve to subtract artifactual AcH formed. However, the correction factor itself was frequently many times larger than AcH values measured for enzymatic formation, resulting in AcH levels near or so close to zero that the data were insignificant. We also observed difficulties in working with the semicarbizide procedure (Stowell 1979). These included many intermediate and tedious steps that were sources of error.

In our desire to improve upon existing methods' accuracy and reproducibility, we first considered the sample source. Because of the reactivity of AcH, it is presumed that AcH concentrations may vary widely in different tissues of the body (Forsander et al. 1969). In blood it is unlikely that AcH forms a common aldehyde pool as in the case of ethanol, where blood levels accurately reflect ethanol concentration in most tissues of the body (Forsander and Sekki 1974). Since most of the oxidation processes for ethanol and AcH take place in the liver (Lieber and DeCarli 1970) and since a fixed proportion of these metabolites reach the lungs directly from the liver, breath could best reflect AcH levels in arterial blood. Furthermore, AcH readily penetrates membranes and equilibrates rapidly with alveolar air (Forsander and Sekki 1974). Accordingly, in early experiments with a technique to capture and concentrate volatile components of breath for direct analysis, it was determined that this method was sufficiently sensitive to measure endogenous (i.e., nondrinking) levels of AcH and ethanol. This finding gave impetus to a refinement of this new method.

Because cigarette smoking can be an important covariable among individuals and because acetaldehyde in cigarette smoke could contribute to alterations in blood and breath levels of AcH and ethanol, we compared breath analyses from fasting smokers with and without a history of alcohol abuse and from nonalcoholic nonsmokers. We were unable to identify in our sample population sufficient numbers of alcoholics who do not smoke cigarettes.

Materials and Methods

Breath Sampling Procedure

The principle employed in this procedure involves freezing out and trapping those volatile components of breath that have a freezing point greater than the temperature of liquid nitrogen. The device is shown in figure 1. The subject inhales and places his mouth firmly around a 2-cm diameter polypropylene mouthpiece. Two seconds later an air pump (DeVilbliss, No. 561, Somerset, Pennsylvania) is turned on, pulling the subject's breath through the sidearm (B, figure 1) of the sampling device at a rate of approximately 10 l per minute. Teflon three-way T-shaped stopcocks (C, D) are positioned to allow breath passage through the sampling loop (c). Sidearms A and B and the bypass are constructed from Pyrex glass tubing (1/4-inch, O.D.) The bottom third of the sampling loop is constructed of 3/8-inch (O.D.) Pyrex glass tubing that contains small glass beads serving to increase the cooled surface area inside the loop. Since the sampling loop is submersed in liquid nitrogen prior to and during the breath collection period, all volatile components of the breath freeze out onto the surface of the glass beads. The remainder of the breath exits the sampling device through A, travels through the pump, and is returned to body temperature by passing through a series of Ehrlenmeyer flasks submersed in a water bath at 37°C. The breath sample then flows to a Medishield Wright respirometer (BOC International LTD, Harlow, England) via 1-inch (O.D.) tubing for volume measurement. Sampling time takes approximately 9 seconds for 1.50 l of breath. After turning the pump off, stopcocks are rotated to the bypass position. The sampling loop is removed from the apparatus and stored in liquid nitrogen until time of analysis. Once the breath sample has been frozen in the sampling loop, very few factors will affect the sensitivity of the method, given standardized GC conditions. To maintain this sensitivity, we find it necessary to have scrupulously clean glassware by washing with detergent and acid, followed by copious rinsing with distilled water.

Sample Analysis

The principle of analysis is to first remove the sample from the sampling device and collect it at the head of the cooled gas chromatography (GC) column. To accomplish this reproducibly, an analytical apparatus was designed as depicted in figure 2. Prior to the connection of the sampling device, the carrier gas is allowed to flow through the line bypass for GC baseline stabilization (figure 2). Lines G and H are an extension of the carrier gas line prior to entering the GC injection block. These tubes are constructed of 1/4-inch (O.D.) Teflon tubing, and all connections are made with Teflon fittings. While the sampling loop is still immersed in liquid nitrogen, the sampling device is connected into the analytic circuit. Stopcocks E and F are rotated, allowing the carrier gas to flow through the tube bypass. After purging the tube bypass, the oven temperature of the GC is lowered from 140°C to 35°C. Stopcocks C and D are then rotated, allowing the carrier gas to pass through the sampling loop. The sampling loop is then removed from the liquid nitrogen, suspended in room air for 2 minutes, then submersed in an oil bath (120°C), where it remains for 5 minutes. During this period, the sample is flushed from the sampling tube and deposited at the head of the GC column. At the end of this period, stopcocks E and F are rotated so that the carrier gas again flows through the line bypass.

Gas Chromatographic Procedures

With a sample at the head of the column, the column oven is rapidly heated to 90°C and allowed to remain at this temperature for 8 minutes. The oven temperature is then programed at a rate of 32°C per minute to a final temperature of 140°C, where it remains for 8 minutes. A Perkin-Elmer 3920 gas chromatograph equipped with dual flame ionization detectors (FID) was used. It contained a 6-foot glass column (1/4-inch O.D. and 4 mm I.D.) packed with 80/100 mesh Poropak Q (Supelco, Belafonte, Pennsylvania). The helium gas flow rate was 40 ml per minute. Air and hydrogen flow rates for the FID were approximately 300 ml and 30 ml per minute, respectively. Injection port and detector temperatures were 175°C and 250°C, respectively. The signal from the FID was transduced onto an Omniscribe chart recorder.

Standard curves were constructed by injecting a series of known quantities of redistilled AcH (ICN Pharmaceuticals, Plainview, New York) and ethanol (Pharmaco Publicker, Inc., Linfield, Pennsylvania) into the sampling loop, followed by analysis as described above. A daily standard of AcH was injected into the GC to assess column viability and GC working parameters. This process also generated a daily correction factor, which was applied to minor shifts in the standard curves.

To determine percent recovery of AcH and ethanol, known quantities of redistilled AcH and ethanol were injected into the sampling loop containing a fasting breath sample. Recordings were compared with those following injection of an equivalent amount of AcH or ethanol directly into the injection port of the GC. Mean percent recoveries for ethanol and AcH were 95.3 percent (90.5 to 98.5 percent) and 93.8 percent (91.0 to 95.0 percent), respectively. Recoveries for methanol and acetone were not determined.

Alcohol subjects (Feighner et al. 1972) were recruited from the alcohol treatment program at the University of Connecticut Health Center, and nonalcoholic smokers and nonsmokers were recruited from the medical school community. All alcoholic subjects were free of ingested ethanol for at least 3 days prior to the study, and all subjects fasted and did not smoke for at least 8 hours prior to breath sampling.

Human Studies: Alcohol Drinking Studies

For subjects consuming an oral dose of ethanol, the same overnight fasting schedule was observed. Thus, when a subject arrived at the laboratory, three breath samples were collected 5 minutes apart prior to consumption of an ethanol solution. For the present study, one subject was asked to consume 1 g ethanol/kg body weight, diluted fivefold with diet ginger ale. This chilled "cocktail" of 20 percent v/v ethanol was divided into three equal portions for consumption over three consecutive 5-minute periods. Breath samples were collected, beginning 15 minutes after consumption of the final portion of the "cocktail," during which time constant rinsing of the mouth with water was done. This latter procedure was required to assure minimal contamination of breath samples by ethanol and AcH retained and/or produced in the mouth. These conditions were previously determined by experiments in which a volunteer maintained a 20 percent solution of ethanol in his mouth for 5 minutes, followed by constant rinsing with water and intermittent breath samples.

Results

Endogenous Levels

From fasting breath samples taken on all subjects, four elutants—methanol, AcH, ethanol, and acetone—were always present. These four elutants were identified on chromatograms by retention time and by internal standards (figure 3). The order of elutants was as follows: methanol (5.0 minutes); AcH (7.5 minutes); ethanol (12.0 minutes); and acetone (15.0 minutes). A series of additional peaks was observed that showed considerable subject variability, but these peaks did not affect the resolution of the four identified elutants. Although other investigators have identified at least 100 volatile components in human breath (Krotoszyski et al. 1977), it was not our present purpose to identify these additional peaks. Thus, concentrating on the AcH component of the breath sample, in a cohort of nonsmoking, nonalcoholic subjects endogenous AcH levels ranged from 0.5 to 11.0 ng/l (table 1). Given this small sampling of a Caucasian population, no apparent influence of age or sex on endogenous AcH levels was observed.

Distributions of endogenous AcH levels among each of the three cohorts are shown in figure 4 and are expressed in nanograms per liter. The mean and standard deviation for nonalcoholic nonsmokers is 4.3 ± 3.3 ng/l (N = 14). This value is similar to an independent evaluation of breath volatile organics by one other laboratory upon a similar cohort (Krotoszyski et al. 1977). Mean AcH with standard deviations for alcoholic and nonalcoholic smokers are 24.5 ± 8.2 and 16.1 ± 9.6 ng/l, respectively.

Mean breath AcH levels for the cohort of 16 alcoholic smokers are significantly higher ($t_{28} = 9.076$; $p < 0.0001$) than mean levels for the cohort of 14 nonalcoholic nonsmokers (table 1). No overlap of values for these two cohorts is apparent (figure 1). By inspection, the AcH values for these two cohorts fit Gaussian distributions (see figure 4). However, the distribution of AcH values for the nonalcoholic smokers clearly is not Gaussian, suggesting heterogeneity in this cohort (figure 4). Retrospective drinking and smoking histories on this cohort did not distinguish high-AcH from low-AcH individuals. Accordingly, there is no evidence that would suggest that the six high-AcH nonalcoholic smokers, as shown in figure 4, represent preclinical or subclinical cases of alcoholism.

Test of Reproducibility

One experimental subject (female) was consecutively sampled at 5-minute intervals for a total of seven breath samples. For these repetitive samples, a mean AcH of 7.2 ± 1.0 (S.D.) ng/l was found, with a coefficient of variation of 0.14. The same female individual sampled over a 4-week interval gave the following values in nanograms per liter: 8.4, 6.0, 7.3, and 7.3 (each value representing the mean of triplicate breath samples at each week). Two male subjects studied over similar weekly intervals displayed comparable degrees of variability.

Breath Acetaldehyde and Ethanol Levels Following Consumption of Alcohol

Within minutes of consuming a 20 percent (w/v) ethanol "cocktail," peak heights of the principal volatile breath components were increased greatly above the endogenous levels (figure 5). We report the results from a male subject consuming a "cocktail" at the final dose of 1.0 gm/kg. Up to 45 minutes after beginning ethanol consumption, the subject's breath AcH increased at a steady rate and reached a plateau of 2.60 μg/l. This value for AcH corresponded to a peak ethanol concentration of 650 μg/l.

Discussion

AcH is formed by enzymic oxidation of ethanol in the liver, then broken down to acetic acid and water by the action of aldehyde dehydrogenase (Kricka and Clark 1979). Hence, consumption of ethanol is promptly followed by a rise in the concentration of AcH in the blood and the breath.

However, even in nonalcoholic individuals drinking no ethanol, a small amount of ethanol is detectable in the blood, probably resulting from bacterial fermentation in the intestine (Krebs and Perkins 1970). Therefore, endogenously generated AcH should be constantly present in the blood, probably in amounts too small to be dependably detected by blood AcH assays in current use. One previous study reported the detection of endogenous AcH in breath (Krotoszyski et al. 1977), and precautions were also taken to limit prior ethanol intake. A mean breath AcH level of approximately 6 ng/l reported by Krotoszyski and coworkers (1977) is similar to our findings (table 1).

Our new technique reproducibly detected AcH in breath with a higher degree of sensitivity than any other method known to us. Using a 1.50 l breath sample, we could detect as little as 10 picomoles of AcH. Interestingly, with this system there is no theoretical limit to the size of the breath sample collected and subsequently concentrated. Thus, the sensitivity of this method can still be increased severalfold.

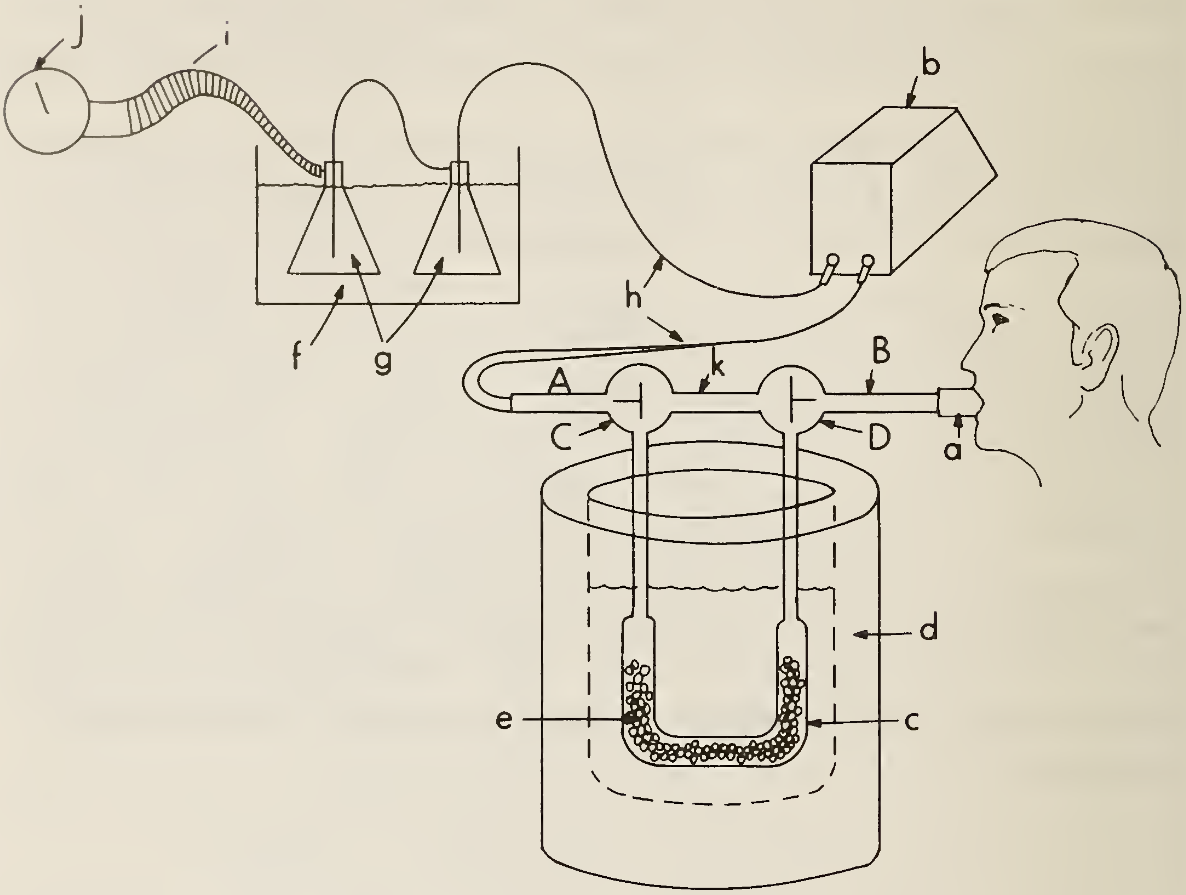
It is evident that sensitivity and reproducibility also depend upon the human subject's ability to consistently replicate breath sampling procedures. Accordingly, rigid experimental conditions were followed, and all subjects were given typewritten instructions before breath sampling and allowed to practice the procedure until they were proficient. Thus, with careful standardization of the technique of sample collection, mean AcH values for consecutive samples on the same day or over several weeks showed a variation of less than 15 percent.

Using our breath assay method, we were able to routinely detect endogenously generated AcH and ethanol in the breath of subjects abstaining from alcoholic beverages, readily measuring levels down to 0.5 ng/l. In addition, we were able to document the time course of rising breath AcH levels in subjects drinking a dose of ethanol without subjecting these volunteers to venipunctures. Given the experimental conditions, observed increases in breath AcH levels most likely reflect metabolic conversion of ingested ethanol rather than associated phenomena (e.g., generation of AcH by microflora of the respiratory tract). These results are consistent with the findings of Stowell et al. (1980) relating breath AcH concentration to enzymatic metabolism of ethanol. However, the validity of current techniques for measuring blood AcH levels is still in doubt; thus, quantitative comparisons between blood and breath AcH cannot yet be made with confidence.

Using this method to study endogenous ethanol and AcH in the breath of alcoholics, we discovered that chronic cigarette smoking is a confounding variable. Since cigarette smoking may be a significant covariable among alcoholics, AcH in cigarette smoke should also be considered a contributor to the pathogenesis of this disease. Although the U.S. Surgeon General's Report of 1972 (U.S. Department of Health, Education, and Welfare 1972) gives AcH in cigarette smoke as a potentially harmful substance, significant physiological deficits from combined ethanol and cigarette abuse have only recently been documented (Sprince et al. 1979). Thus, additive effects of aldehyde from ethanol metabolism and cigarette smoking may have adverse effects not only on pulmonary function, but also on hepatic, nervous, and cardiovascular systems (Sprince et al. 1979).

Our findings of elevated endogenous levels of ethanol and AcH in the breath of alcoholic and nonalcoholic smokers could be a consequence of altered GI or respiratory microflora. These results could also reflect inherited metabolic differences among these cohorts. Although there is evidence in the literature for decreased acetaldehyde dehydrogenase among alcoholics (Jenkins and Peters 1980) and family members of alcoholics (Goodwin 1976; Stowell et al. 1977; Zeiner et al. 1979), issues of cause and effect need clarification. Family pedigree studies should be helpful in sorting out genetic and environmental determinations of ethanol metabolism. Since breath may represent an optimal sample source for AcH determinations (Dannecker et al. 1981; Stowell et al. 1980), the contribution to AcH levels from chronic cigarette smoking must be considered.

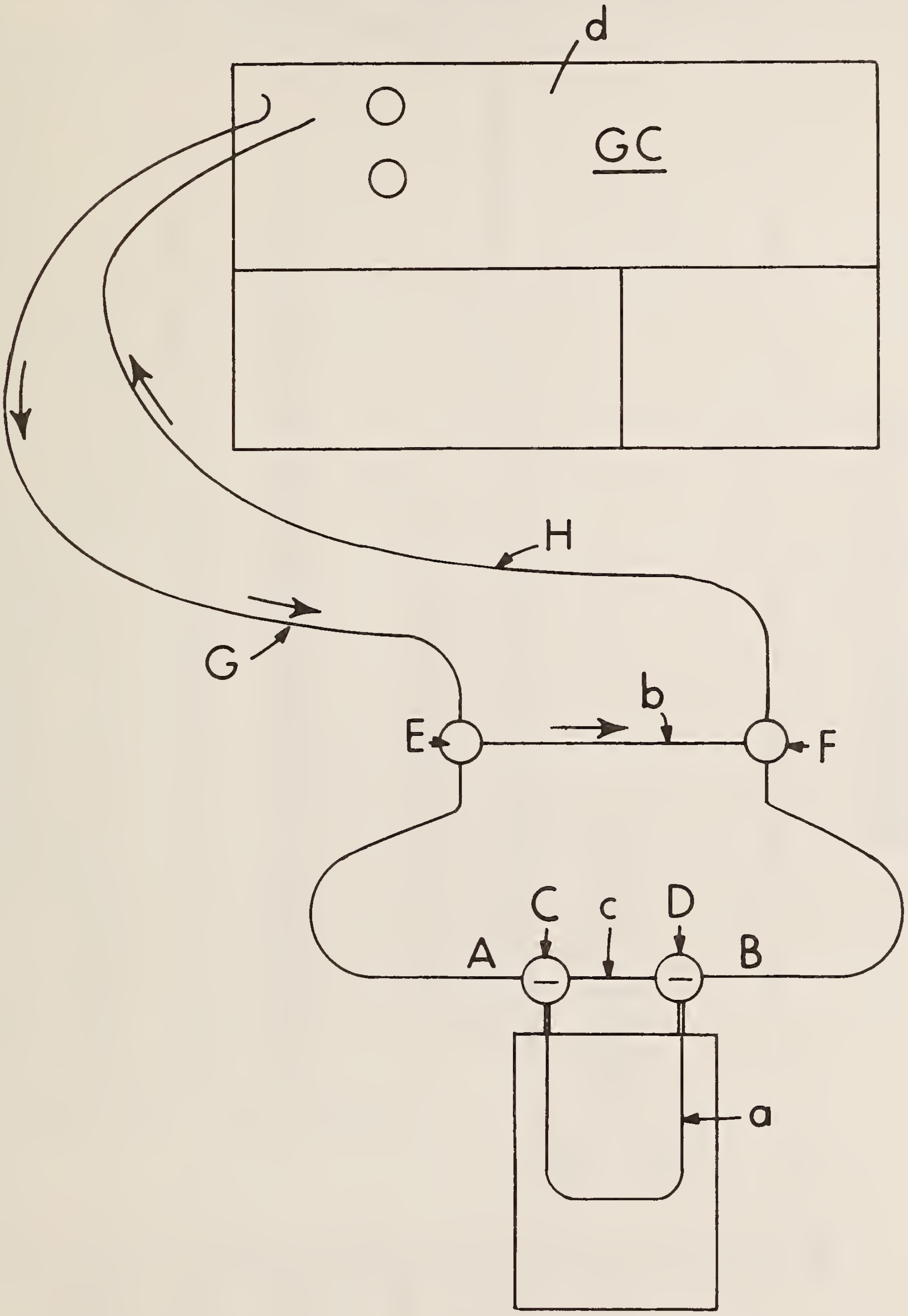
Figure 1. Human Breath Sampling Apparatus



SOURCE: Dannecker, Shaskan, and Phillips. *Anal Biochem*, 114:2, 1981. Copyright 1981 by Academic Press, Inc.

Note: (a) mouth piece; (b) pump; (c) sampling loop; (d) Dewar flask containing liquid nitrogen; (e) glass beads; (f) water bath, 37°C; (g) Erlenmeyer flasks; (h) Tygon tubing; (i) 1-inch (O.D.) plastic tubing; (j) Wright respirometer; (k) bypass; (A) side arm A; (B) side arm B; (C) stopcock C; (D) stopcock D.

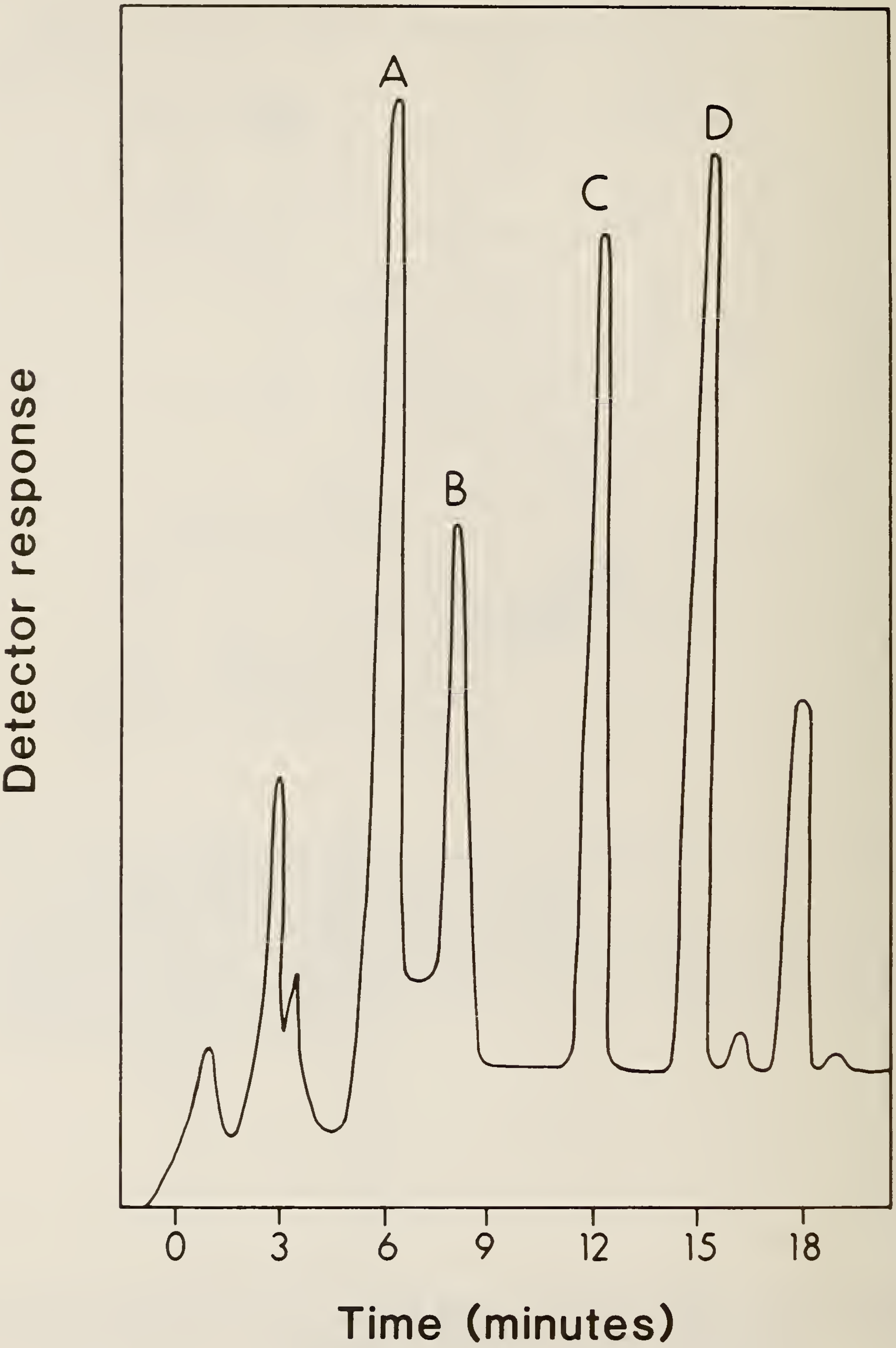
Figure 2. Breath Sampling Device Connected to Gas Chromatography for Sample Analyses



SOURCE: Dannecker, Shaskan, and Phillips. *Anal Biochem*, 114:2, 1981. Copyright 1981 by Academic Press, Inc.

Note: (a) sampling loop; (b) line bypass; (c) tube bypass; (d) gas chromatograph; (A) side arm A; (B) side arm B; (C) stopcock C; (D) stopcock D; (E) stopcock E; (F) stopcock F; (G) extension of GC carrier gas line.

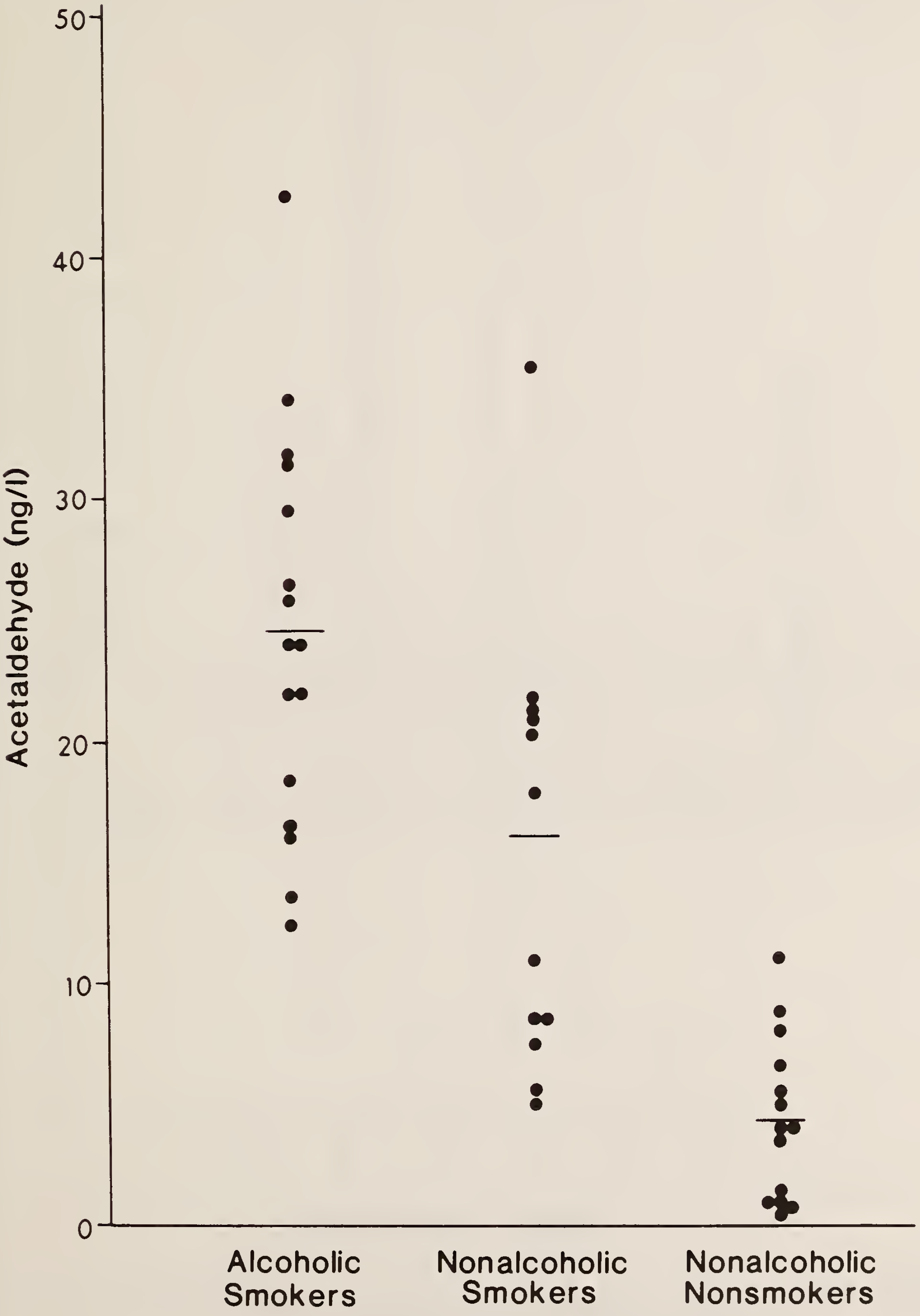
Figure 3. **Representative Chromatogram Showing Endogenous Levels of Volatile Compounds in 1.50 Liter of Breath From a Fasting Human Subject**



SOURCE: Dannecker, Shaskan, and Phillips. *Anal Biochem*, 114:4, 1981. Copyright 1981 by Academic Press, Inc.

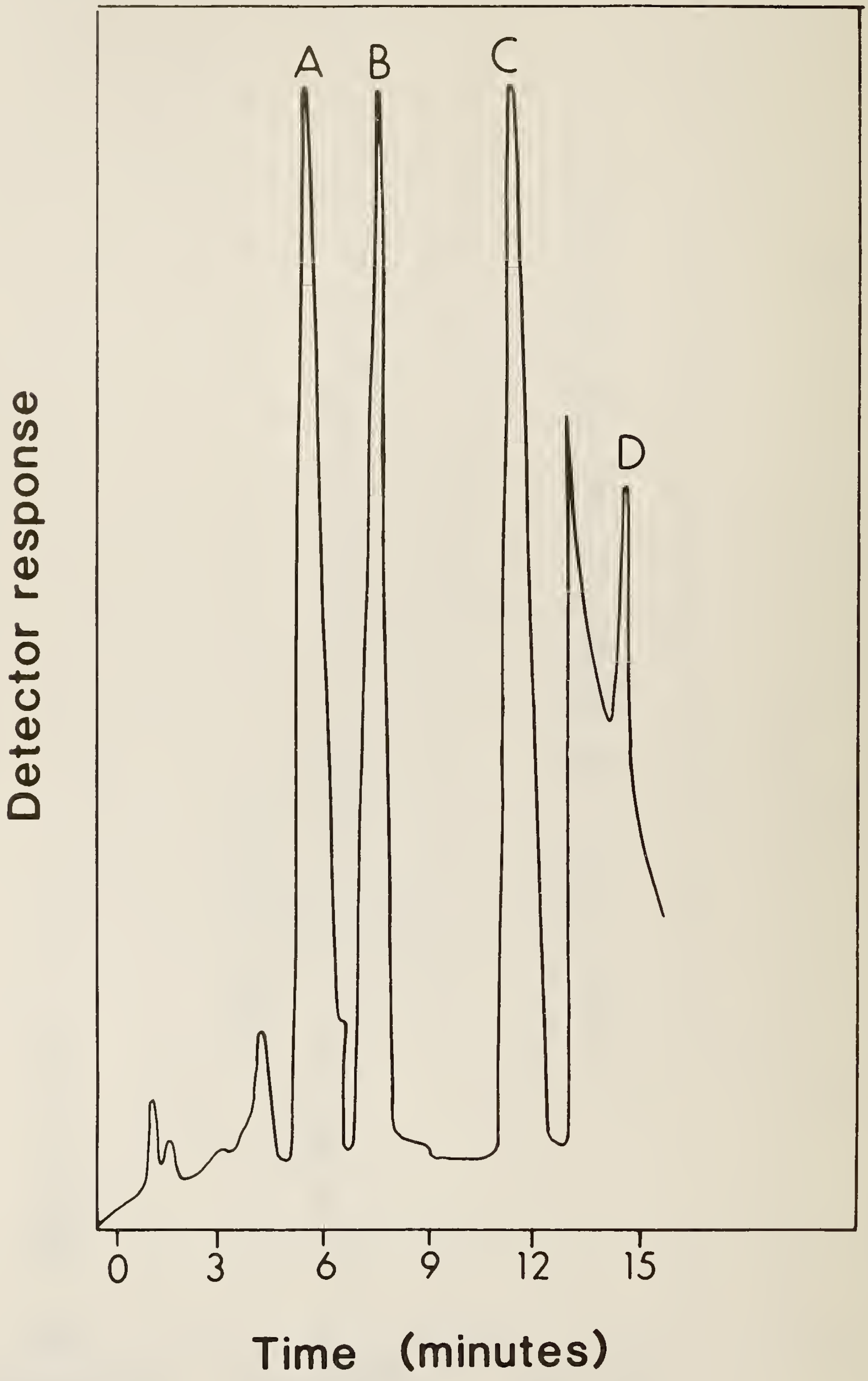
Note: (A) methanol, X 8 attenuation; (B) acetaldehyde, X 8; (C) ethanol, X 64; (D) acetone, X 256.

Figure 4. Distribution of Endogenous Breath Acetaldehyde Levels Among Alcoholic Smokers (N = 16), Nonalcoholic Smokers (N = 12), and Nonalcoholic Nonsmokers (N = 14)



Note: Each data point represents the mean value for triplicate breath samples taken from fasting individuals. The bar represents the arithmetic mean value for each cohort.

Figure 5. Chromatogram Showing Levels of Volatile Compounds in 1.50 l of Breath From a Human Subject After Ethanol Consumption (1.0 gm/kg)



SOURCE: Dannecker, Shaskan, and Phillips. *Anal Biochem*, 114:5, 1981. Copyright 1981 by Academic Press, Inc.

Note: (A) methanol, X 16 attenuation; (B) acetaldehyde, X 256; (C) ethanol, X 51200; (D) acetone, X 512.

Table 1. Breath Endogenous Acetaldehyde Levels Among Human Subjects

Research Subject No.	Age	Sex	Acetaldehyde ng/l
3506	19	M	3.3
3502	23	M	8.8
3501	27	M	6.7
3515	28	M	8.0
3505	32	M	1.5
3504	37	M	5.3
3503	40	M	11.0
3508	42	M	0.5
3510	66	M	1.0
3507	18	F	4.0
3512	20	F	1.0
3511	27	F	4.0
3509	49	F	0.7
3516	55	F	5.0

SOURCE: Dannecker, Shaskan, and Phillips. *Anal Biochem*, 114:4, 1981. Copyright 1981 by Academic Press, Inc.

Note: Subjects were nonsmoker, nonalcoholic Caucasian adults. Breath samples were taken after a minimum of an 8-hour fast, as described in text.

References

Cederbaum, A.I.; Lieber, C.S.; and Rubin, E. Effects of chronic ethanol treatment on mitochondrial functions damage to coupling site I[1]. *Arch Biochem Biophys*, 165:560-569, 1974.

Dannecker, J.R., Jr.; Shaskan, E.G.; and Phillips, M. A new highly sensitive assay for breath acetaldehyde: Detection of endogenous levels in humans. *Anal Biochem*, 114:2-5, 1981.

Eriksson, C.J.P. Problems and pitfalls in acetaldehyde determinations. *Alcohol Clin Exp Res*, 4:22-29, 1980.

Eriksson, C.J.P.; Sippel, H.W.; and Forsander, O.A. The determination of acetaldehyde in biological samples by head-space chromatography. *Anal Biochem*, 80:116-124, 1977.

Feighner, J.P.; Robins, E.; Guze, S.B.; Woodruff, R.A.; Winokur, G.; and Mung, R. Diagnostic criteria for use in psychiatric research. *Arch Gen Psychiatry*, 26:57-63, 1972.

Forsander, O.A., and Sekki, A. Acetaldehyde and ethanol in the breath of rats after alcohol administration. *Med Biol*, 52:276-280, 1974.

Forsander, O.A.; Hillborn, M.E.; and Lindros, K.O. Influence of thyroid function on the acetaldehyde level of blood and liver of intact rats during ethanol metabolism. *Acta Pharmacol Toxicol*, 27:410-416, 1969.

Goodwin, D. *Is Alcoholism Hereditary*? New York: Oxford University Press, 1976.

Jenkins, W.J., and Peters, T.J. Selectively reduced hepatic acetaldehyde dehydrogenase in alcoholics. *Lancet*, 1:628-629, 1980.

Korsten, M.A.; Matsuzaki, S.; Feinman, L.; and Lieber, C.S. High blood acetaldehyde levels after ethanol administration. *N Engl J Med*, 292:386-389, 1975.

Krebs, H.A., and Perkins, J.R. The physiological role of liver alcohol dehydrogenase. *Biochem J*, 118:635-644, 1970.

Kricka, L.J., and Clark, P.M.S. *Biochemistry of Alcohol and Alcoholism*. Chichester, England: Ellis Howood Ltd., Halsted Press, 1979.

Krotoszyski, B.; Gabriel, G.; O'Neill, H.; and Claudio, W.P.A. Characterization of human expired air: A promising investigation and diagnostic technique. *J Chromatogr Sci*, 15:239-244, 1977.

Lieber, C.S., and DeCarli, L.M. Hepatic microsomal ethanol-oxidizing system: In vitro characteristics and adaptive properties in vivo. *J Biol Chem*, 245:2505-2512, 1970.

Schuckit, M.A., and Rayses, V. Ethanol ingestion: Differences in blood acetaldehyde concentrations in relatives of alcoholics and controls. *Science*, 203:54-55, 1979.

Sprince, H.; Parker, C.M.; and Smith, G.F. Comparison of protection by L-asorbic acid, L-cysteine and adrenergic blocking agents against acetaldehyde, acrotein, and formaldehyde. *Agents Actions*, 9:407-414, 1979.

Stowell, A.R.; Greenway, R.M.; and Batt, R.D. Acetaldehyde formation during deproteinization of human blood samples containing ethanol. *Biochem Med*, 18:392-401, 1977.

Stowell, A.R. An improved method for the determination of acetaldehyde in human blood with minimal ethanol interference. *Clin Chim Acta*, 98:201-205, 1979.

Stowell, A.R.; Lindros, K.O.; and Salaspuro, M.P. Breath and blood acetaldehyde concentrations and their correlation during normal and calcium carbimide-modified ethanol oxidation in man. *Biochem Pharmacol*, 29:783-787, 1980.

U.S. Department of Health, Education, and Welfare. *The Health Consequences of Smoking, A Report to the Surgeon General: 1972.* DHEW Pub. No. (HSM)72-7516. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1972.

Zeiner, A.R.; Paredis, A.; and Christensen, H.D. The role of acetaldehyde in mediating reactivity to an acute dose of ethanol among different racial groups. *Alcohol Clin Exp Res,* 3:11-18, 1979.

Elevation of Acetaldehyde Levels After Chronic Alcohol Consumption: Pathogenesis and Pathologic Consequences*

Charles S. Lieber, Enrique Baraona, Ellen R. Gordon, Pekka Jauhonen, M.E. Lebsack, and Pekka Pikkarainen

Acetaldehyde, the first product of all known pathways of hepatic oxidation, normally does not accumulate and is rapidly oxidized by aldehyde dehydrogenase (ALDH) to acetate. Despite its known reactivity, acetaldehyde has been neglected because of methodological difficulties. Recent technological advancements have permitted the development of precise, sensitive methods to detect acetaldehyde in biological fluids. As a result, several studies have been carried out that have shown a profound involvement of this compound in the development of alcoholic liver injury.

Pathways of Acetaldehyde Metabolism

It is now generally accepted that more than 90 percent of the acetaldehyde formed from ethanol is oxidized in the liver to acetate by ALDH (Lindros 1974). Racker (1949) was the first to report the presence of NAD-linked ALDH in bovine liver. Subsequent investi-

---

Note: Figures and table appear at end of paper.

*Original studies described in this paper were supported, in part, by U.S. Public Health Service grants AA 03508 and AA 07275 and the Medical Research Service of the Veterans Administration. The paper was presented at the 19th annual meeting of the American College of Neuropsychopharmacology, San Juan, Puerto Rico.

gators detected ALDH activity in mitochondria (Walkenstein and Weinhouse 1953), cytoplasm (Büttner 1965; Deitrich 1966), and microsomes (Deitrich 1966; Tietz et al. 1964; Tottmar et al. 1973) of rat liver. These various dehydrogenases have been characterized by their differential affinities for the substrate and electrophoretic properties (Grunnet 1973; Koivula and Koivusalo 1975; Lundquist et al. 1962; Marjanen 1973; Tottmar et al. 1973; Weiner et al. 1974).

In rat liver, Smith and Packer (1972) and Tottmar et al. (1973), using kinetic analysis, obtained evidence for two mitochondrial enzymes. Subsequent investigations (Koivula and Koivusalo 1975; Siew et al. 1976) substantiated these findings. One of these isoenzymes, designated ALDH I, has a K_m for acetaldehyde of approximately 1 M. Isoenzyme II has a K_m of about 1 mM. In addition, isoenzyme II can also function with NADP as a cofactor and may be identical to the microsomal enzyme.

Current understanding of the number and properties of ALDH isozymes has been facilitated by the use of various drugs that induce specific isozymes of ALDH. Redmond and Cohen (1971) noted that phenobarbital treatment results in a twofold increase in ALDH activity in 700 g supernatant fraction of mouse liver homogenates. Deitrich (1971) observed that mitochondrial ALDH activity was unaffected by phenobarbital treatment, but a tenfold induction was detected in the cytosolic ALDH activity in some strains of rats. The induction is controlled by a single gene displaying incomplete dominance. Subsequent studies of the phenobarbital induction (Deitrich et al. 1972) support the concept that the cytosol contains one ALDH isozyme that is induced by phenobarbital and another that is not; each ALDH isoenzyme exhibits different substrate specificity. More recently, evidence for a third cytosolic ALDH isozyme has been found that was induced up to 100-fold by 2,3,7,8,-tetrachlorodibenzo-p-dioxin (TCDD). This isozyme does not appear to exist in all strains of rats (Roper et al. 1976).

The question of the subcellular site of acetaldehyde metabolism has been approached by several authors (Corrall et al. 1976; Grunnet 1973; Parrilla et al. 1974), all of whom conclude that in the rat, the principal site of acetaldehyde metabolism is the mitochondria. This conclusion is logical, since isozyme I, which is found in the mitochondrial matrix, has the greatest affinity for acetaldehyde. Indeed, after ethanol administration, acetaldehyde levels in rat liver are very low (Eriksson 1973; Kesäniemi 1974), suggesting that the major portion of acetaldehyde oxidation occurs in the mitochondrial matrix where the low K_m enzyme is located. Cytosolic ALDH, however, could play a minor role in acetaldehyde metabolism, since induction of one cytosolic enzyme by phenobarbital in susceptible strains of rats resulted in lower blood acetaldehyde levels after large doses of ethanol (Petersen et al. 1977). Furthermore, from experimental studies using tritiated ethanol in isolated hepatocytes, it was calculated that 20 percent of the acetaldehyde formed from ethanol is metabolized in the cytosol (Grunnet et al. 1976).

Whether the results obtained in rodents and the derived conclusions apply to other species (including humans) is the subject of debate. Only limited studies have been carried out in primates, primarily in human autopsy samples, suggesting the existence of one (Kraemer and Deitrich 1968), two (Greenfield and Pietruszko 1977), four (Harada et al. 1980), or five (Koivula 1975) forms of ALDH in human liver. Recently, it was found that baboon liver has detectable ALDH activity in the mitochondrial, microsomal, and cytosolic fractions. Based on kinetic data, the mitochondria and the cytosol each appear to contain two isozymes of ALDH, one with a high and the other with a low K_m for acetaldehyde, and in the microsomes there is a form with high K_m only. In baboon liver, more than 60 percent of total ALDH activity resides in the mitochondria (Lebsack et al. in press).

Hasumura et al. (1975, 1976) found that isolated, intact rat liver mitochondria oxidize acetaldehyde at a rate of approximately 15 nmol/min/mg protein at 30°C with 180 M acetaldehyde as substrate, and consume oxygen at the same rate. This reaction was markedly enhanced by modifiers of the mitochondrial respiratory chain, including ADP, which increased the rate of acetaldehyde oxidation by 88 percent. 2,4-Dinitrophenol, an uncoupler of the mitochondrial respiratory chain, also stimulated the oxidation of acetaldehyde in both isolated mitochondria and in perfused livers (Eriksson 1973). By contrast, rotenone, an inhibitor of mitochondrial respiration at site I, virtually abolished acetaldehyde oxidation. As reported in isolated hepatocytes (Parrilla et al. 1974), acetaldehyde oxidation in the isolated mitochondria was also diminished by the addition of substrates for mitochondrial NAD-linked dehydrogenases. Since the ADP:O ratio with 180 μM acetaldehyde was 2.6, a ratio similar to that with glutamate as substrate, it is also likely that acetaldehyde oxidation is coupled with oxidative phosphorylation. Indeed, little acetaldehyde is oxidized in mitochondria in which membranes are disrupted by sodium deoxycholate. These results indicate that the oxidation of acetaldehyde in liver mitochondria is linked to the mitochondrial respiratory chain at the site of NAD-linked dehydrogenases.

Dietary factors may also alter the rate of the oxidation of acetaldehyde. Indeed, it has been noted that rats maintained on some commercial diets have increased blood acetaldehyde levels associated with a decreased ALDH activity. It was demonstrated that one diet contained a derivative of cyanamide, a potent inhibitor of ALDH (Marchner and Tottmar 1976). Low-protein diets can also reduce ALDH activity (Lindros et al. 1977) but do not necessarily cause an increase in blood acetaldehyde levels (Lindros and Hillbom 1979).

Effect of Chronic Ethanol Consumption on Acetaldehyde Metabolism and Blood Acetaldehyde Levels

Alterations in Acetaldehyde Metabolism

Chronic ethanol consumption results in a significant reduction in the capacity of isolated rat liver mitochondria to oxidize acetaldehyde (Hasumura et al. 1975). It was reported, however, that chronic ethanol consumption did not change (Raskin and Sokoloff 1972; Redmond and Cohen 1971; Tottmar et al. 1974) or even increase (Dajani et al. 1963; Horton 1971) the activity of hepatic NAD-dependent ALDH. In all of these reports, the enzyme activity was assayed utilizing very high concentrations of acetaldehyde (between 3 μM and 12 μM) as substrate. These levels are several orders of magnitude higher than the acetaldehyde concentrations observed in the liver after ethanol administration (Baraona et al. 1979; Eriksson 1973; Kesäniemi 1974). Even so, more recent studies, in which more appropriate levels of substrate were utilized, do not resolve these discrepancies. It has been reported that the activity of the NAD-dependent ALDH with high affinity for acetaldehyde remained unchanged (Hasumura et al. 1976; Koivula and Lindros 1975), increased (Horton and Barrett 1976), or decreased following chronic ethanol consumption (Koivula and Lindros 1975; Lebsack et al. in press). The discrepancy between the decreased rate of acetaldehyde oxidation in intact mitochondria and the inconsistently affected in vitro enzyme activity suggests that the rate-limiting step of acetaldehyde oxidation is the mitochondrial capacity to reoxidize NADH rather than ALDH activity (Hasumura et al. 1975).

The decreased capacity of mitochondria of alcohol-fed animals to oxidize acetaldehyde, associated with unaltered or even enhanced rates of ethanol oxidation (and therefore acetaldehyde generation), may result in an imbalance between production and disposition of acetaldehyde. Such a mechanism may result in the elevated acetaldehyde levels observed after chronic ethanol consumption in humans (Korsten et al. 1975), in rats (Baraona et al. 1979; Koivula and Lindros 1975), and in baboons (Pikkarainen et al. in press). In the baboon, plasma "free" acetaldehyde correlated positively with production rate of acetaldehyde ($r = 0.69$, $p < 0.001$) and negatively with liver mitochondrial ALDH activity ($r = -0.59$, $p < 0.01$). When these determinants of blood acetaldehyde were combined (acetaldehyde production rate/ALDH activity), a correlation coefficient of 0.84 ($p < 0.001$) resulted, suggesting that in addition to increased production, decreased catabolism may contribute to the higher acetaldehyde levels seen after chronic ethanol consumption (Pikkarainen et al. in press). One hypothesis that might explain, at least in part, the increased level of acetaldehyde proposes a relative shift of the oxidation of acetaldehyde from the low K_m mitochondrial pathway to the high K_m cytosolic one. Such a shift might be anticipated, in view of the dependence of the mitochondrial pathway on mitochondrial integrity and the known alterations of mitochondrial functions after chronic ethanol consumption, including decreased acetaldehyde catabolism. If a greater proportion is processed via nonmitochondrial systems, one would expect that a higher steady-state concentration of acetaldehyde would be required to maintain the same total flux of acetaldehyde, in view of the much higher K_m of the nonmitochondrial systems. Enhancement of the effect might be expected if the total flux of acetaldehyde is increased (because of accelerated ethanol metabolism).

The higher liver acetaldehyde might be expected to result in higher hepatic vein acetaldehyde. The latter in turn would elevate peripheral blood acetaldehyde. Blood acetaldehyde levels also depend on peripheral metabolism, which can be altered (Lambouef et al. 1980). In any event, evidence of changes in blood acetaldehyde levels has been slow in forthcoming. Truitt (1971) studied acetaldehyde levels after oral administration of ethanol to alcoholics and nonalcoholics and found no statistically significant differences. Freund and O'Hollaren (1965) found a plateau of acetaldehyde concentration in human alveolar air after ethanol administration, but the relation to ethanol metabolism was not clarified. Although Majchrowicz and Mendelson (1970) also found elevated levels in alcoholics, they related this finding to the amount of acetaldehyde contained as a congener in alcoholic beverages because there appeared to be no relationship between blood ethanol and acetaldehyde concentrations in their clinical study. Magrinat et al. (1975) reached a similar conclusion.

More recently, Korsten et al. (1975) found a relationship between blood acetaldehyde and ethanol levels in humans after intravenous administration of ethanol yielding concentrations high enough to saturate hepatic ethanol oxidizing systems, ADH as well as MEOS. This study revealed that the acetaldehyde level remained relatively constant despite wide variations in blood ethanol above 30 mM, but the acetaldehyde plateau abruptly terminated when ethanol concentration reached about 22 mM, a concentration at which non-ADH pathways are not saturated. Moreover, the plateau level of acetaldehyde was significantly higher in alcoholics than in nonalcoholics. This difference in blood acetaldehyde between alcoholics and nonalcoholics was considered to a consequence of chronic ethanol consumption. However, in addition to the metabolic causes for the elevation of blood acetaldehyde in the alcoholic, discussed before, a genetic influence has been postulated (Schuckit and Rayses 1979). After a test dose of alcohol, blood acetaldehyde levels were significantly higher in relatives of alcoholics than in matched controls. The methodology of acetaldehyde measurement, however, has been questioned (Eriksson 1980*a*).

Acetaldehyde Determinations in Blood and Expired Air

Two problems have plagued the measurement of acetaldehyde in biological fluids: the rapid disappearance of acetaldehyde from the blood and the artifactual production of acetaldehyde from ethanol (Eriksson 1980*b*). When proteins are precipitated in the presence of ethanol, there is a major nonenzymatic production of acetaldehyde, particularly in the presence of red cells. Recent methodologic advances partially alleviate these two problems.

The disappearance of acetaldehyde from whole blood could represent binding to red blood cells (Gaines et al. 1977) or metabolism of acetaldehyde in the blood cells or in the plasma. It has recently been shown that in rat blood there is an uneven distribution of acetaldehyde between blood cells and plasma, with about 75 percent in the cells (Eriksson et al. 1977; Tottmar et al. 1978). However, in human blood no binding to red blood cells has been shown (Eriksson et al. 1977). Our in vitro distribution studies, using water-hemolyzed washed red blood cells, also showed that the amount of acetaldehyde bound to red blood cells is minimal (Pikkarainen et al. 1979). Recently, ALDH activity was found in erythrocytes (Inoue et al. 1978; Pietruszko and Vallari 1978), and this activity could also be responsible for the acetaldehyde disappearance, at least during and immediately after the withdrawal of the blood while it is still at 37°C. One method for blood acetaldehyde measurement proposes to eliminate this problem by inhibiting the ALDH activity with chloral hydrate (von Wartburg 1979). Another recently published method uses semicarbazide to trap the acetaldehyde in the plasma obtained by rapid centrifugation (Stowell 1979). The purpose of semicarbazide as well as rapid centrifugation is to avoid the disappearance of acetaldehyde from the blood.

Furthermore, by carrying out the acid precipitation in the plasma in the absence of red blood cells, the spontaneous formation of acetaldehyde from ethanol is minimized and a smaller correction is required. We have presented a new method for the measurement of "free" acetaldehyde in plasma obtained by rapid centrifugation. By this procedure, spontaneous formation of acetaldehyde from ethanol is almost totally eliminated by avoiding the precipitation of protein (Pikkarainen et al. 1979). Indeed, the artifactual production of acetaldehyde from ethanol is 20 to 50 times less with this method (as well as with the semicarbazide or chloral hydrate methods) than with the whole blood precipitation procedure (Stowell et al. 1977). In addition, the sensitivity of the head-space gas chromatography was improved in the "free" acetaldehyde method by eliminating the dilution of the sample. As a result, a sensitivity of 0.5 M acetaldehyde has been achieved.

The values of blood acetaldehyde found using the new methods are far below the levels in the blood published previously (Kinoshita 1974; Korsten et al. 1975; Truitt 1971), but are similar to those found in cerebrospinal fluid (Lindros and Hillbom 1979). The high levels with the other methods may be partly attributed to the fact that the artifactual formation of acetaldehyde from ethanol was not fully eliminated and/or compensated for by the correction factors. An elevation of plasma acetaldehyde, determined by an improved method, was found after chronic ethanol consumption in baboons (Pikkarainen et al. in press).

In view of the difficulties in blood acetaldehyde measurement, breath acetaldehyde has been used as an index of blood acetaldehyde (Freund and O'Hollaren 1965; Fukui 1969; Zeiner et al. 1979). To study to what extent this index is justified, we measured ethanol and acetaldehyde concentrations in right atrium and peripheral blood of catheterized baboons (while awake) and in breath samples collected through an endotracheal tube or a facial mask (Jauhonen et al. in press). In the tube samples, breath acetaldehyde was only 16 percent of that in the mask samples, whereas ethanol was much higher in the tube air than in the mask air. Alveolar acetaldehyde calculated from the tube acetaldehyde levels correlated significantly with those in right atrium and peripheral blood. Contrasting with air obtained through the endotracheal tube, acetaldehyde collected through the mask did not correlate with and greatly overestimated blood acetaldehyde levels. A partition factor of 211±13 was found between blood acetaldehyde (measured with the semicarbazide method) and alveolar air acetaldehyde. This factor did not differ significantly from the value of 190 used by other authors (Stowell et al. 1980).

In human volunteers given ethanol, acetaldehyde in end-expiratory samples yielded a twofold to sixfold overestimation of blood acetaldehyde concentrations, particularly in smokers. This overestimation may be explained by the active oxidation of ethanol to acetaldehyde in the upper respiratory tract, and the resulting increase in the acetaldehyde concentration in areas through which end-expiratory air passes in the sampling process (Pikkarainen et al. 1981). Indeed, introduction of ethanol into the oral cavity resulted in rapid formation of acetaldehyde (figure 1). Incubation of saliva with ethanol also produced acetaldehyde in vitro (figure 2). This activity could be removed by filtration and was inhibited by pyrazole. Moreover, this acetaldehyde production increased after bacteriological culture of the oral content, indicating an active role of microorganisms of the oropharynx (Pikkarainen et al. 1981). This contribution of the upper respiratory tract was only slightly diminished by taking end-expiratory samples. The overestimation was almost completely abolished by rinsing the mouth with the ADH inhibitor pyrazole, at least in nonsmokers; it was less inhibited in smokers (table 1). In this regard, it is of interest to note that isolated rat lung microsomes produce acetaldehyde from ethanol (figure 3) and that this effect is enhanced by chronic alcohol consumption (figure 4) (Pikkarainen et al. 1981). It is tempting to speculate that smoking increases lung acetaldehyde production by inducing microsomal ethanol oxidation, but direct evidence of this is lacking. In conclusion, at blood acetaldehyde levels in the low micromolar range, calculations based on measurement of total breath acetaldehyde usually overestimate blood levels. Under these conditions, breath acetaldehyde correlates poorly with blood acetaldehyde concentrations.

Effects Attributable to Acetaldehyde After Acute and Chronic Ethanol Consumption

The metabolic effects of acetaldehyde remain unclear, even though it has been speculated that this compound may contribute to the complications of alcoholism (Truitt and Duritz 1966) and to the numerous toxic effects noted in this disease (Walsh 1971). Acetaldehyde stimulates the release of catecholamines (Eade 1959); this action as well as some direct effects of acetaldehyde on blood vessels might be responsible for the flushing of the skin sometimes observed after alcohol ingestion. In some Orientals, even small amounts of alcohol that have almost no effect on Caucasians can produce a facial flush (Wolff 1973), a sensitivity that appears to be genetically determined. The mechanism of this action has not been elucidated. This effect has been attributed to an unusually high initial elevation of blood acetaldehyde after ethanol ingestion (Ijiri 1974). The Oriental population in which the flush is common is also characterized by a high incidence of "atypical ADH." It has been speculated that in Orientals the intake of ethanol is followed by a burst of ethanol oxidation due to the high enzyme activity of the "atypical ADH" (von Wartburg 1977) before the reoxidation of NADH becomes rate limiting in the ADH pathway. One could postulate that this oxidation causes an outpouring of acetaldehyde from the liver, resulting in the flush. Experimental support for this hypothesis, however, is scant. An alternative hypothesis has been proposed by Harada et al. (1979), who suggest that the flushing in Orientals may be caused by the lack of one of the aldehyde dehydrogenases. In any event, among Orientals with flushing, there was a tendency toward higher blood acetaldehyde levels (Mizoi et al. 1979), but the difference was not statistically significant in all studies (Ewing et al. 1974). In our studies, three Japanese with flushing had clearly higher acetaldehyde concentrations (up to over 100 M) than one nonflushing Japanese volunteer who had low acetaldehyde concentrations similar to those usually observed in Caucasians (Pikkarainen et al. unpublished data). Ojibwa Indians were also found to have higher blood acetaldehyde levels than Caucasians, but in one study Chinese did not (Reed et al. 1976), and breath acetaldehyde was increased in another (Zeiner et al. 1979). The flushing reaction seen in susceptible Orientals resembles the disulfiram reaction caused by the elevation of acetaldehyde resulting from ALDH inhibition.

As previously reviewed (Lieber 1977), during ethanol consumption the metabolism of some amines (such as serotonin and norepinephrine) shifts from conversion to the acid to the production of the reduced (alcoholic) compound. The major reason for this change appears to be the competitive inhibition of the corresponding ALDH by acetaldehyde. In addition, acetaldehyde has been shown to participate in and to favor condensation reactions of biogenic amines (Cohen and Collins 1970; Davis and Walsh 1970). The products of these interactions have been shown to appear in the urine of alcoholics (Collins and Nijon 1979), and they have been postulated to have addictive properties if sufficient amounts were generated in vivo. Furthermore, some of the condensation products may be hepatotoxic (Moura et al. 1977). Alterations in CNS amine levels have been reported after administration of acetaldehyde in experimental animals at doses that achieve blood concentrations comparable to those found after intravenous alcohol administration. Moreover, withdrawal symptoms were observed when acetaldehyde administration was discontinued. These symptoms were similar to those occurring during alcohol withdrawal syndromes (Ortiz et al. 1974). On the other hand, Amir (1978) noted that brain ALDH activity increased following prolonged ethanol administration. This would tend to reduce brain acetaldehyde after chronic ethanol consumption. The fate of acetaldehyde in the brain and at interactions with CNS metabolites has as yet not been clearly defined and is the subject of controversy.

Acetaldehyde can affect many other tissues. Myocardial protein synthesis is impaired by acetaldehyde (Schreiber et al. 1972, 1974). This effect might contribute to the development of alcoholic cardiomyopathy. Morphologic changes in erythrocytes caused by acetaldehyde binding have also been reported (Gaines et al. 1977). In the liver, acetaldehyde may act as a hepatotoxic agent by promoting lipid peroxidation (Shaw et al. in press) and by covalently binding to proteins (Nomura and Lieber 1980) (figure 5). Of particular interest is the binding of acetaldehyde to tubulin, the constituent protein of microtubules. Indeed, liver microtubules have recently been recognized as another possible site for acetaldehyde toxicity (Matsuda et al. 1979). In isolated hepatocytes, ethanol significantly decreased polymerized tubulin, and there was also a corresponding reduction of microtubules. This effect of ethanol was reproduced by the addition of acetaldehyde. Hepatic microtubular alterations were also found in vivo after chronic ethanol administration, in association with decreased protein secretion and protein retention in the liver (Baraona et al. 1977). Engorgement of the endoplasmic reticulum (Iseri 1966), Golgi apparatus, and secretory vesicles (Matsuda et al. 1979) with VLDL-like particles suggested a concomitant impairment of lipoprotein secretion.

The rate of ethanol clearance is faster in alcoholics than in nonalcoholics, a possible source of greater production of acetaldehyde. On the other hand, both hepatic ALDH with high affinity for acetaldehyde and the reoxidation of the cofactor NADH to NAD depend on mitochondrial integrity. Both may be impaired by chronic alcohol consumption, resulting in higher acetaldehyde levels in the liver. The latter may in turn enhance the functional disturbance of the mitochondria by reducing the activity of various shuttles involved in the disposition of reducing equivalents and by inhibiting oxidative phosphorylation (Cederbaum et al. 1974). Cederbaum, Lieber, Beattie, and Rubin (1975) have also shown that acetaldehyde depresses the capacity of liver mitochondria to oxidize fatty acids, but concentrations of acetaldehyde required to achieve the effects were greater than those likely to occur in vivo. However, hepatic mitochondria of rats fed ethanol chronically have been found to have an increased susceptibility to the effects of acetaldehyde (figure 6). Under these conditions, concentrations of acetaldehyde that could occur in the liver were found to depress mitochondrial functions (Matsuzaki et al. 1977), including the oxidation of lipids. Thus, acetaldehyde causes mitochondrial dysfunction, which in turn produces higher acetaldehyde levels. This results in a "vicious cycle" that could lead to a progressive increase of acetaldehyde levels and to further damage in the liver (figure 7).

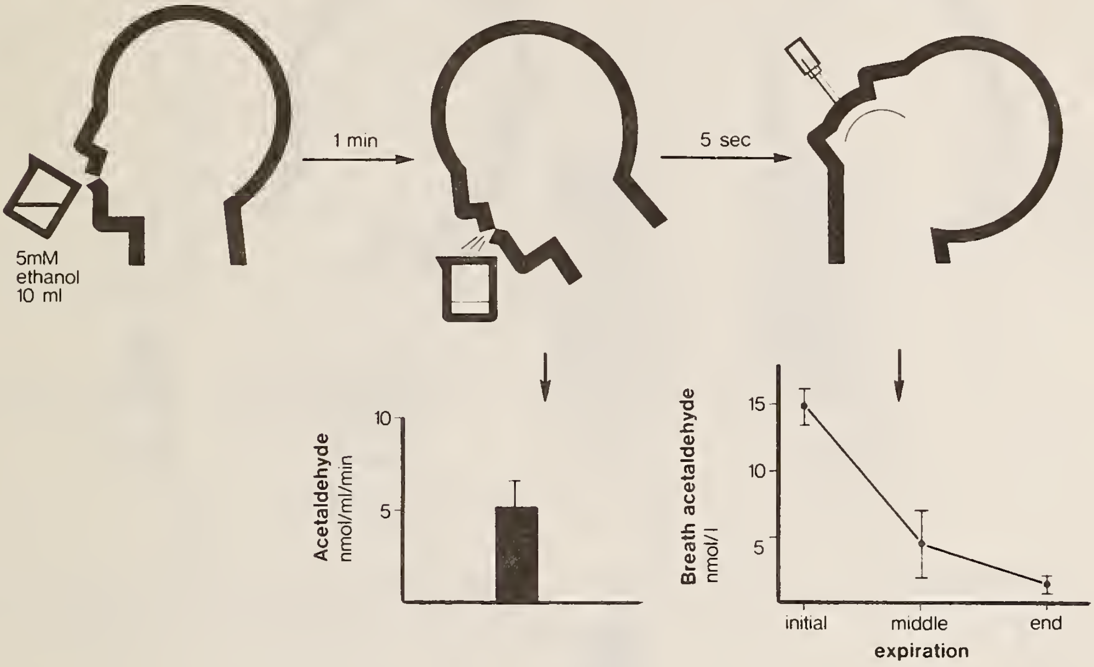
Summary

Progress has been made in the understanding of both acetaldehyde metabolism and its pathologic effects in part because of the

development of new methods for acetaldehyde determination. It has now been established that livers of primates contain two isoenzymes of aldehyde dehydrogenase, one with high and one with low affinity for acetaldehyde, both in the cytosol and the mitochondria. The main catalyst of acetaldehyde oxidation appears to be the mitochondrial low K_m enzyme. Although blood acetaldehyde concentrations during ethanol oxidation are very low (in the micromolar range) in nonalcoholic humans and in naive experimental animals, evidence is now accumulating that after chronic ethanol consumption acetaldehyde concentrations increase. This increase may be due to an accelerated ethanol elimination rate and/or a decreased capacity of the liver to handle acetaldehyde, in part because of mitochondrial damage induced by the acetaldehyde.

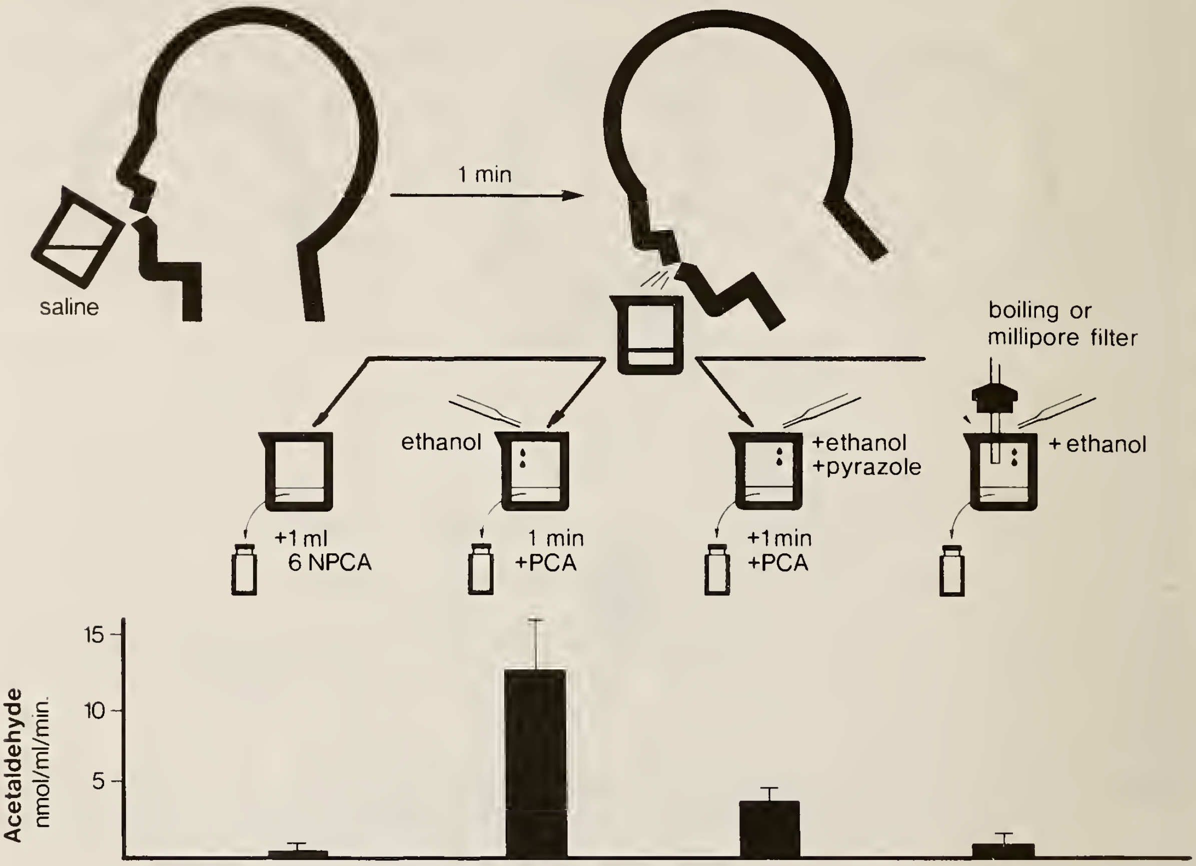
Acetaldehyde can react with and bind to many biologically active compounds (e.g., tubulin, biogenic amines, glutathione), which may contribute to profound pathologic changes in various tissues, including liver, heart, and brain. Recent findings in the respiratory tract also suggest a possible impact of smoking on acetaldehyde metabolism. In addition, acetaldehyde may be involved in the development of dependence and some of the manifestations of intolerance to alcohol exhibited by predisposed individuals.

Figure 1. In Vivo Formation of Acetaldehyde From Ethanol in the Oral Cavity



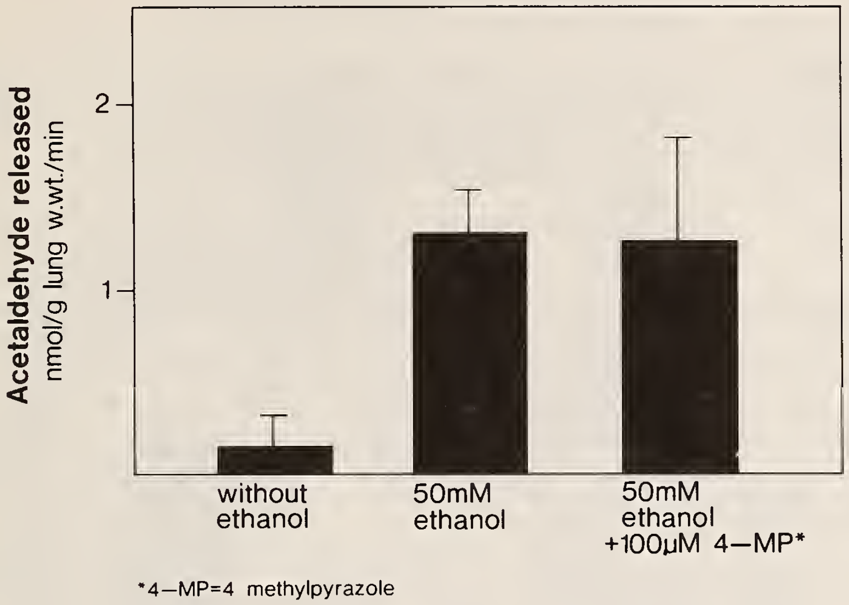
Note: Five volunteers rinsed their mouths with 10 ml of 5 mM ethanol solution in saline for 1 min, and the acetaldehyde produced was measured in the mouthwash by head-space gas chromatography. Immediately after the mouth-rinsing, breath samples (initial, middle, and end-expiratory) for acetaldehyde measurement were taken.

Figure 2. **In Vitro Formation of Acetaldehyde From Ethanol in Saline Mouthwashes**



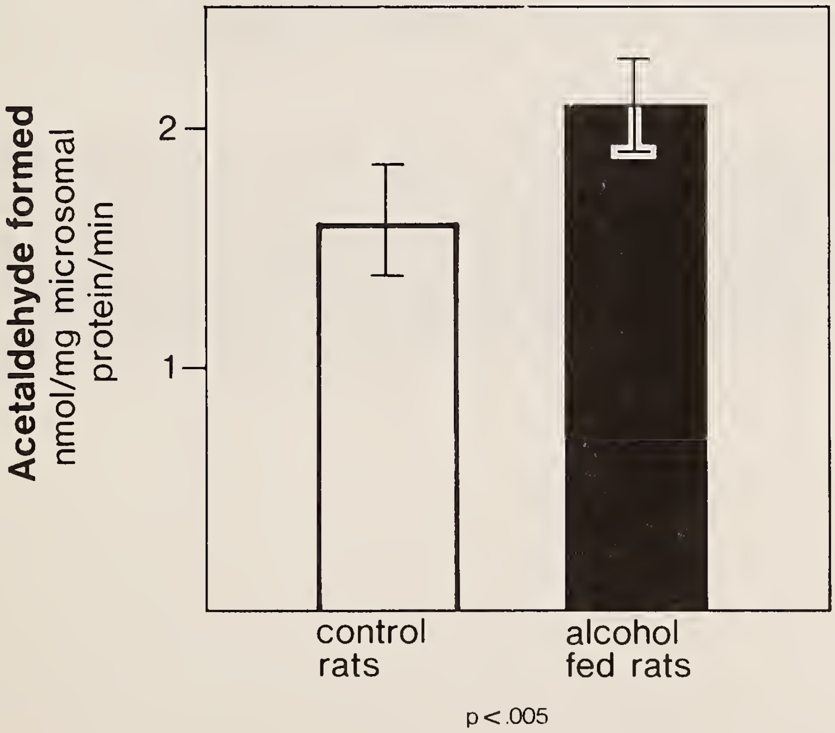
Note: Aliquots of saline mouthwashes from five subjects were incubated with and without 24 mM ethanol for 5 min. The reaction was stopped by addition of perchloric acid (PCA), and acetaldehyde was measured by head-space gas chromatography. Acetaldehyde was produced from ethanol. This was inhibited by 76 percent in the presence of 5 mM pyrazole and completely abolished by boiling (for 10 min) or by millipore filtration of the mouthwashes.

Figure 3. Acetaldehyde Formation in Lung Slices of Naive Rats



Note: Acetaldehyde production from 50 mM ethanol was insensitive to inhibition by 100 μM 4-methylpyrazole.

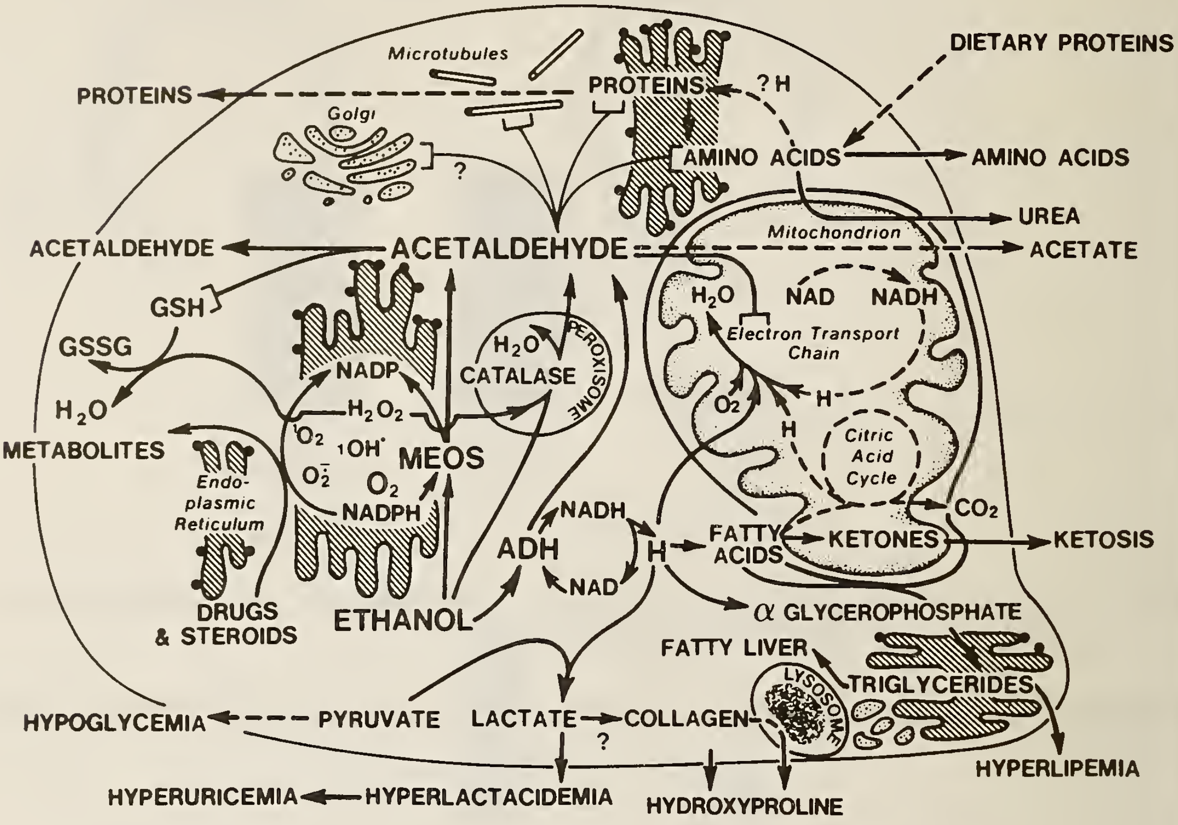
Figure 4. Acetaldehyde Formation in Lung Microsomes



SOURCE: Pikkarainen, Baraona, Jauhonen, Seitz, and Lieber. *J Lab Clin Med*, 97:634, 1981. Copyright 1981 by the C.V. Mosby Company.

Note: Lung microsomes from alcohol-fed and pair-fed control rats were incubated in the presence of 50 mM ethanol. Acetaldehyde production was enhanced by chronic alcohol consumption.

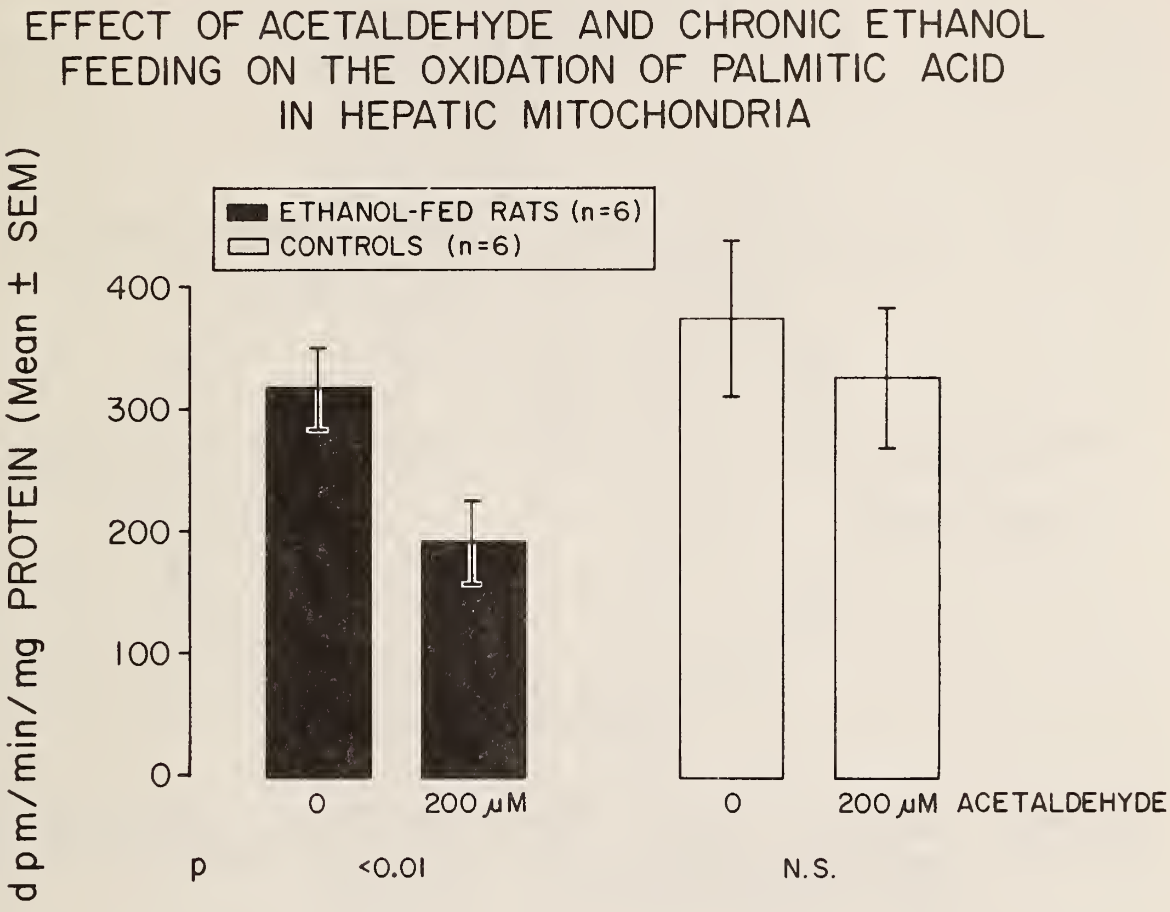
Figure 5. Oxidation of Ethanol in the Hepatocyte and the Role of Acetaldehyde in the Disturbances of Intermediary Metabolism



SOURCE: Lieber. In: Leevy, C. M., ed. *Clinics in Gastroenterology.* London: W.B. Saunders, Ltd., 1981. p. 316. Copyright 1981 by W.B. Saunders Company, Ltd.

Note: The broken lines indicate pathways that are depressed by ethanol. The symbol -{ denotes interference or binding by the acetaldehyde.

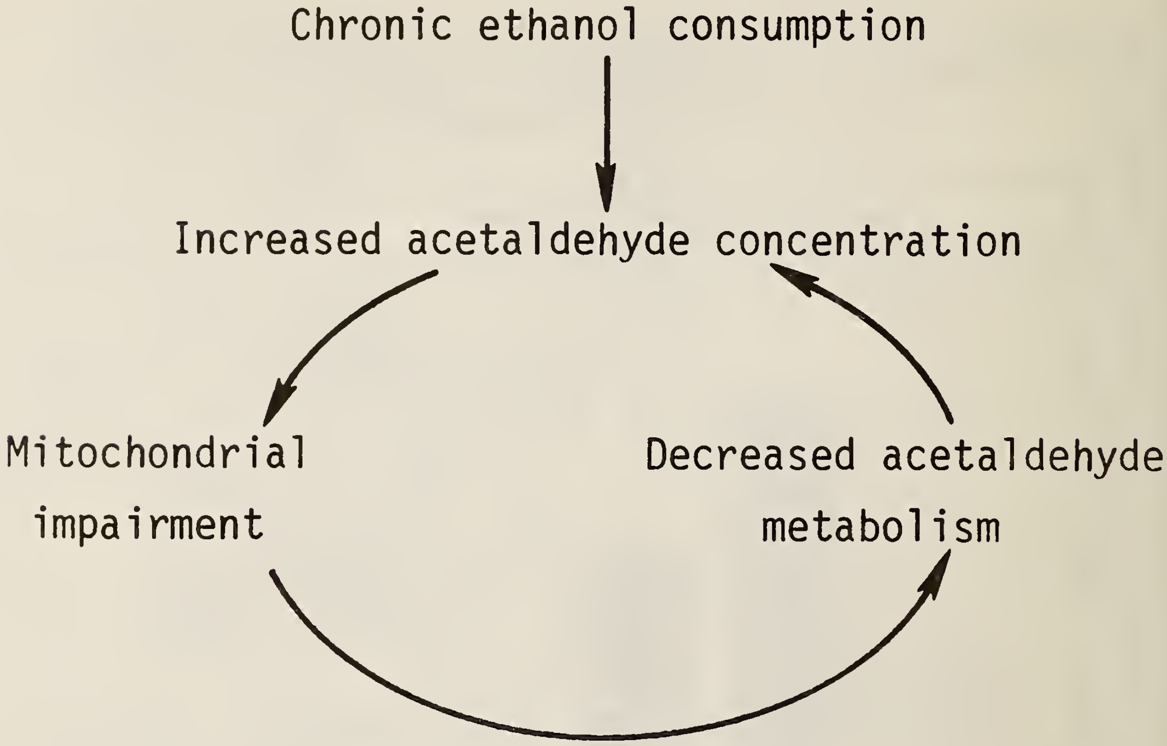
Figure 6. Effect of Chronic Ethanol Feeding and the Presence of Acetaldehyde on Fatty Acid Oxidation in Hepatic Mitochondria



SOURCE: Data from Matsuzaki et al. In: Fisher, M. M., and Rankin, J. G., eds. *Alcohol and the Liver*, Vol. 3. New York: Plenum Press, 1977. p. 132. Copyright 1977 by Plenum Publishing Corp.

Note: Palmitic acid oxidation was studied by measuring CO_2 production using $1\text{-}^{14}C$-labeled palmitic acid.

Figure 7. A Schematic Representation of a Possible Interrelationship Between Ethanol, Acetaldehyde, and Mitochondrial Impairment


Chronic ethanol consumption
Increased acetaldehyde concentration
Mitochondrial impairment
Decreased acetaldehyde metabolism

SOURCE: Hasumura, Teschke, and Lieber. *Science*, 189:727-729, 1975. Copyright 1975 by the American Association for the Advancement of Science.

Table 1. Acetaldehyde Concentrations in Blood and Various Fractions of Expiratory Air 60 min After Ethanol Ingestion (0.8 g/kg) in Smoker and Nonsmoker Volunteers

	Breath Acetaldehyde (nmoles/l)			Blood Acetaldehyde (μmol/l)	
Group	Initial	Middle	End-Expiratory	Measured	Calculated From End-Expiratory Air[a]
Smokers	101 ± 22[b]	91 ± 17[b]	87 ± 20	2.6 ± 0.6	16.5 ± 3.8
Nonsmokers	44 ± 4	33 ± 5	39 ± 7	2.1 ± 0.1	7.4 ± 1.3

[a] Using blood/breath partition factor of 190.

[b] Statistically signficant difference between smokers and nonsmokers, $p < 0.05$.

References

Amir, S. Brain aldehyde dehydrogenase: Adaptive increase following prolonged ethanol administration in rats. *Neuropharmacology*, 17:463-467, 1978.

Baraona, E.; Leo, M.A.; Borowsky, S.A.; and Lieber, C.S. Pathogenesis of alcohol-induced accumulation of protein in the liver. *J Clin Invest*, 60:546-554, 1977.

Baraona, E.; Matsuda, Y.; Pikkarainen, P.; Finkelman, F.; and Lieber, C.S. Exaggeration of ethanol-induced decrease in liver microtubules after chronic alcohol consumption: Role of acetaldehyde. *Gastroenterology*, 76:1274, 1979.

Brunengraber, H.; Boutry, M.; Lowenstein, L.; and Lowenstein, J.M. In: Thurman, R.G.; Yonetani, T.; Williamson, J.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems*. New York: Academic Press, 1973. p. 329.

Büttner, H. Aldehyd- und Alkoholdehydrogenase. Aktivitat in Leber und Niere der Ratte. *Biochem Z*, 341:300-314, 1965.

Cederbaum, A.L.; Lieber, C.S.; and Rubin, E. The effect of acetaldehyde on mitochondrial function. *Arch Biochem Biophys*, 161:26-39, 1974.

Cederbaum, A.L.; Lieber, C.S.; Beattie, D.S.; and Rubin, E. Effect of chronic ethanol ingestion on fatty acid oxidation by hepatic mitochondria. *J Biol Chem*, 250:5122-5129, 1975.

Cederbaum, A.L.; Lieber, C.S.; and Rubin, E. Effect of acetaldehyde on fatty acid oxidation and ketogenesis by hepatic mitochondria. *Arch Biochem Biophys*, 169:29-41, 1975.

Cohen, G., and Collins, M. Alkaloids from catecholamines in adrenal tissue: Possible role in alcoholism. *Science*, 167:1749-1751, 1970.

Collins, M.A., and Nijon, W.P. Dopamine-related tetrahydroisoquinolines: Significant urinary excretion by alcoholics after chronic alcohol consumption. *Science*, 206:1184-1186, 197 .

Corrall, R.J.M.; Havre, P.; Margolis, J.; Kong, M.; and Landau, R. Subcellular site of acetaldehyde oxidation in rat liver. *Biochem Pharmacol*, 25:17-20, 1976.

Dajani, R.M.; Danielski, J.; and Orten, J.M. The utilization of ethanol. II. The alcohol-acetaldehyde dehydrogenase systems in the livers of alcohol-treated rats. *J Nutr*, 80:196-204, 1963.

Davis, V.E., and Walsh, M.J. Alcohol, amines and alkaloids: Possible biochemical basis for alcohol addiction. *Science*, 167:1005-1007, 1970.

Deitrich, R.A. Tissue and subcellular distribution of mammalian aldehydeoxidizing capacity. *Biochem Pharmacol*, 15:1911-1922, 1966.

Deitrich, R.A. Genetic aspects of increase in rat liver aldehyde dehydrogenase induced by phenobarbital. *Science*, 173:334-336, 1971.

Deitrich, R.A.; Collins, A.C.; and Erwin, V.G. Genetic influence upon phenobarbital-induced increase in rat liver supernatant aldehyde dehydrogenase activity. *J Biol Chem*, 247:7232-7236, 1972.

Eade, N.R. Mechanisms of sympathomimetic action of aldehydes. *J Pharmacol Exp Ther*, 127:29-34, 1959.

Eriksson, C.J.P. Ethanol and acetaldehyde metabolism in rat strains genetically selected for their ethanol preference. *Biochem Pharmacol*, 22:2283-2292, 1973.

Eriksson, C.J.P. Elevated blood acetaldehyde levels in alcoholics and their relatives: A reevaluation. *Science*, 207:1383-1384, 1980*a*.

Eriksson, C.J.P. Problems and pitfalls in acetaldehyde determinations. *Alcohol Clin Exp Res*, 4:22-29, 1980*b*.

Eriksson, C.J.P.; Sippel, H.W.; and Forsander, O.A. The occurrence of acetaldehyde binding in rat blood but not in human blood. *FEBS Let*, 75:205-208, 1977.

Ewing, J.A.; Rouse, B.A.; and Pellizzari, E.D. Alcohol sensitivity and ethnic background. *Am J Psychiatry*, 131:206-210, 1974.

Freund, G., and O'Hollaren, P. Acetaldehyde concentrations in alveolar air following a standard dose of ethanol in man. *J Lipid Res*, 6:471-477, 1965.

Fukui, Y. Gas chromatograph determination of acetaldehyde in the expired air after ingestion of alcohol. *Jpn J Leg Med*, 23:24, 1969.

Gaines, K.C.; Salhany, J.M.; Tuma, D.J.; and Sorrell, M.F. Reaction of acetaldehyde with human erythrocyte membrane proteins. *FEBS Lett*, 75:115-119, 1977.

Greenfield, N.J., and Pietruszko, R. Two aldehyde dehydrogenases from human liver. Isolation via affinity chromatography and characterization of the isozymes. *Biochim Biophys Acta*, 483:35-45, 1977.

Grunnet, N. Oxidation of acetaldehyde by rat-liver mitochondria in relation to ethanol oxidation and the transport of reducing equivalents across the mitochondrial membrane. *Eur J Biochem*, 35:236-243, 1973.

Grunnet, N.; Thieden, H.I.D.; and Quistorff, B. Metabolism of 1-^{3}H-ethanol by isolated liver cells. Time-course of the transfer of tritium from R, S-1-^{3}H-ethanol to lactate and β-hydroxybutyrate. *Acta Chem Scand*, B30:345-352, 1976.

Harada, S.; Agarwal, D.P.; and Goedde, H.W. Electrophoretic and biochemical studies of human aldehyde dehydrogenase isozymes in various tissues. *Life Sci*, 26:1773-1780, 1980.

Hasumura, Y.; Teschke, R.; and Lieber, C.S. Acetaldehyde oxidation by hepatic mitochondria. Decrease after chronic ethanol consumption. *Science*, 189:727-729, 1975.

Hasumura, Y.; Teschke, R.; and Lieber, C.S. Characteristics of acetaldehyde oxidation in rat liver mitochondria. *J Biol Chem*, 251:4908-4913, 1976.

Horton, A.A. Induction of aldehyde dehydrogenase in a mitochondrial fraction. *Biochim Biophys Acta*, 253:514-517, 1971.

Horton, A.A., and Barrett, M.C. Rates of induction of mitochondrial aldehyde dehydrogenase in rat liver. *Biochem J*, 156:177-179, 1976.

Ijiri, I. Studies of the relationship between the concentrations of blood acetaldehyde and urinary catecholamine and the symptoms after drinking alcohol. *Jpn J Stud Alcohol*, 9:35-39, 1974.

Inoue, K.; Ohbora, Y.; and Yamasawa, K. Metabolism of acetaldehyde by human erythrocytes. *Life Sci*, 23:179-184, 1978.

Iseri, O. The clinicopathological proteins of the alcoholic liver injury in Japan and its etiological relationship to hepatitis B virus. *Gastroenterology* (Japan), 12:230-240, 1966.

Jauhonen, P.; Baraona, E.; and Lieber, C.S. Contribution of acetaldehyde production in upper respiratory tract to breath acetaldehyde measurements. *Alcohol Clin Exp Res*, in press.

Kesäniemi, Y.A. Metabolism of ethanol and acetaldehyde in intact rats during pregnancy. *Biochem Pharmacol*, 23:1157-1162, 1974.

Kinoshita, M. Gas chromatographic determination of blood acetaldehyde after ingestion of ethanol in man. *Jpn J Stud Alcohol*, 9:1-9, 1974.

Koivula, T. Subcellular distribution and characterization of human liver aldehyde dehydrogenase fractions. *Life Sci*, 16:1563-1570, 1975.

Koivula, T., and Koivusalo, M. Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. *Biochim Biophys Acta*, 397:9-23, 1975.

Koivula, T., and Lindros, K.O. Effects of long term ethanol treatment on aldehyde and alcohol dehydrogenase activities in rat liver. *Biochem Pharmacol*, 24:1937-1942, 1975.

Korsten, M.A.; Matsuzaki, S.; Feinman, L.; and Lieber, C.S. High blood acetaldehyde levels after ethanol administration in alcoholics. Difference between alcoholic and non-alcoholic subjects. *N Engl J Med*, 292:386-389, 1975.

Kraemer, R.J., and Deitrich, R.A. Isolation and characterization of human liver aldehyde dehydrogenase. *J Biol Chem*, 243:6402-6408, 1968.

Lamboeuf, Y.; de Saint Blanquat, G.; and Derache, R. Effect of withdrawal from alcohol on extra-hepatic alcohol and aldehyde dehydrogenase activities in the rat. *Experientia*, 36:286-288, 1980.

Lebsack, M.E.; Gordon, E.R.; and Lieber, C.S. The effect of chronic ethanol consumption on aldehyde dehydrogenase activity in the baboon. *Biochem Pharmacol*, in press.

Lieber, C.S. Metabolism of ethanol. In: Lieber, C.S., ed. *Metabolic Aspects of Alcoholism*. Baltimore: University Park Press, 1977. pp. 1-29.

Lieber, C.S. Metabolic effects of ethanol on the liver and other digestive organs. In: Leevy, C.M., ed. *Clinics in Gastroenterology*. London: W.B. Saunders Co., Ltd., 1981.

Lindros, K.O. Acetaldehyde oxidation and its role in the overall metabolic effects of ethanol in the liver; in regulation of hepatic metabolism. In: Lundquist, F., and Tygstrup, N., eds. *Proceedings of the Alfred Benson Symposium. VI. Copenhagen*(1973). Copenhagen: Munksgaard, 1974. pp. 417-432.

Lindros, K.O., and Hillbom, M.E. Acetaldehyde in cerebrospinal fluid: Its near-absence in ethanol-intoxicated alcoholics. *Med Biol*, 57:246-247, 1979.

Lindros, K.O.; Pekkanen, L.; and Koivula, T. Effect of a low-protein diet on acetaldehyde metabolism in rats. *Acta Pharmacol Toxicol*, 40:134-144, 1977.

Lundquist, F.; Fugmann, U.; Rasmussen, H.; and Svendsen, L. The metabolism of acetaldehyde in mammalian tissue. *Biochem J*, 84:281-286, 1962.

Magrinat, G.; Dolan, J.P.; Biddy, R.L.; Miller, L.D.; and Korol, B. Ethanol and methanol metabolites in alcohol withdrawal. *Nature*, 244:234-235, 1975.

Majchrowicz, E., and Mendelson, J.H. Blood concentration of acetaldehyde and ethanol in chronic alcoholics. *Science*, 168:1100-1102, 1970.

Marchner, H., and Tottmar, O. Inhibition of the acetaldehyde dehydrogenases in rat liver by a cyanamide derivative present in a commercial standard diet for small animals. *Acta Pharmacol Toxicol*, 39:331-343, 1976.

Marjanen, L. Comparison of aldehyde dehydrogenases from cytosol and mitochondria of rat liver. *Biochim Biophys Acta*, 327:238-246, 1973.

Matsuda, Y.; Baraona, E.; Salaspuro, M.; and Lieber, C.S. Effects of ethanol on liver microtubules and Golgi apparatus. *Lab Invest*, 41:455-463, 1979.

Matsuzaki, S., and Lieber, C.S. Increased susceptibility of hepatic mitochondria to the toxicity of acetaldehyde after chronic ethanol consumption. *Biochem Biophys Res Commun*, 75:1059-1065, 1977.

Matsuzaki, S.; Teschke, R.; Ohnishi, K.; and Lieber, C.S. Acceleration of ethanol metabolism by high ethanol concentrations and chronic ethanol consumption: Role of the microsomal ethanol oxidizing system (MEOS). In: Fisher, M.M., and Rankin, J.G., eds. *Alcohol and the Liver*. Vol. 3. New York: Plenum Press, 1977. pp. 119-143.

Mizoi, Y.; Ijiri, I.; Tatsuno, Y.; Kijima, T.; Fujiwara, S.; and Adachi, J. Relationship between facial flushing and blood acetaldehyde levels after alcohol intake. *Pharmacol Biochem Behav*, 10:303-311, 1979.

Moura, D.; Azevedo, L.; and Osswald, W. Hepatotoxicity of the condensation product of adrenaline with acetaldehyde. *J Pharm Pharmacol*, 29:255-256, 1977.

Nomura, F., and Lieber, C.S. Binding of acetaldehyde to rat liver microsomes: Enhancement after chronic alcohol consumption. *Gastroenterology*, 79:1117, 1980.

Ortiz, A.; Griffiths, P.J.; and Littleton, J.A. A comparison of the effects of chronic administration of ethanol and acetaldehyde to mice: Evidence for a role of acetaldehyde in ethanol dependence. *J Pharm Pharmacol*, 26:249-260, 1974.

Parrilla, R.; Ohkawa, K.; Lindros, K.O.; Zimmerman, J.P.; Kopayashe, K.; and Williamson, J.R. Functional compartmentation of acetaldehyde oxidation in rat liver. *J Biol Chem*, 249:4926-4933, 1974.

Petersen, D.; Collins, A.C.; and Deitrich, R.A. Role of liver cytosolic aldehyde dehydrogenase isozymes in control of blood acetaldehyde concentrations. *J Pharmacol Exp Ther*, 201:471-481, 1977.

Pietruszko, R., and Vallari, R.C. Aldehyde dehydrogenase in human blood. *FEBS Lett*, 92:89-91, 1978.

Pikkarainen, P.H.; Baraona, E.; Jauhonen, P.; Seitz, H.K.; and Lieber, C.S. Contribution of oropharynx microflora and of lung microsomes to acetaldehyde in expired air after alcohol ingestion. *J Lab Clin Med*, 97:634, 1981.

Pikkarainen, P.H.; Gordon, E.R.; Lebsack, M.E.; and Lieber, C.S. Determinants of plasma free acetaldehyde level during the steady state oxidation of ethanol: Effects of chronic ethanol feeding. *Biochem Pharmacol*, in press.

Pikkarainen, P.H.; Salaspuro, M.P.; and Lieber, C.S. A method for the determination of "free" acetaldehyde in plasma. *Alcohol Clin Exp Res*, 3:259-261, 1979.

Racker, E. Aldehyde dehydrogenase, a diphosphopyridine nucleotide linked enzyme. *J Biol Chem*, 177:883-892, 1949.

Raskin, N.H., and Sokoloff, L. Ethanol-induced adaptation of alcohol dehydrogenase activity in rat brain. *Nature*, 236:138-140, 1972.

Redmond, G., and Cohen, G. Induction of liver acetaldehyde dehydrogenase: Possible role in ethanol tolerance after exposure to barbiturates. *Science*, 171:387-389, 1971.

Reed, T.E.; Kalant, H.; Gibbins, R.J.; Kapur, B.M.; and Rankin, J.G. Alcohol and acetaldehyde metabolism in Caucasians, Chinese and Amerinds. *CMA J*, 115:851-855, 1976.

Roper, M.; Stock, T.; and Deitrich, R.A. Phenobarbital and tetrachlorodibenzo-p-dioxin induce different isoenzymes of aldehyde dehydrogenase. *Fed Proc*, 35:282, 1976.

Schreiber, S.S.; Briden, K.; Oratz, M.; and Rothschild, M.A. Ethanol, acetaldehyde and myocardial protein synthesis. *J Clin Invest*, 51:2808-2819, 9972.

Schreiber, S.S.; Oratz, M.; Rothschild, M.A.; Reff, F.; and Evans, C. Alcoholic cardiomyopathy. II. The inhibition of cardiac microsomal protein synthesis by acetaldehyde. *J Mol Cell Cardiol*, 6:207-213, 1974.

Schuckit, M.A., and Rayses, V. Ethanol ingestion: Differences in blood acetaldehyde concentrations in relatives of alcoholics and controls. *Science*, 203:54-55, 1979.

Seitz, H.K.; Garro, A.J.; and Lieber, C.S. Enhanced pulmonary and intestinal activation of procarcinogens and mutagens after chronic ethanol consumption in the rat. *Eur J Clin Invest*, in press.

Shaw, S.; Jayatilleke, E.; Ross, W.A.; Gordon, E.R.; and Lieber, C.S. Ethanol induced lipid peroxidation: Potentiation by chronic alcohol feeding and attenuation by methionine. *J Lab Clin Med*, in press.

Siew, C.; Deitrich, R.A.; and Erwin, V.G. Localization and characteristics of rat liver mitochondrial aldehyde dehydrogenases. *Arch Biochem Biophys*, 176:638, 1976.

Smith, L., and Packer, L. Aldehyde oxidation in rat liver mitochondria. *Arch Biochem Biophys*, 148:270-276, 1972.

Stowell, A.R. An improved method for the determination of acetaldehyde in human blood with minimal ethanol interference. *Clin Chim Acta*, 98:201-205, 1979.

Stowell, A.R.; Greenway, R.M.; and Batt, R.D. Acetaldehyde formation during deproteinization of human blood samples containing ethanol. *Biochem Med*, 18:393-401, 1977.

Stowell, A.R.; Lindros, K.O.; and Salaspuro, M.P. Breath and blood acetaldehyde concentrations and their correlation during normal and calcium carbimide-modified ethanol oxidation in man. *Biochem Pharmacol*, 29:783-787, 1980.

Tietz, A.; Lindberg, M.; and Kennedy, E.P. A new pteridine-requiring enzyme system for the oxidation of glyceryl ethers. *J Biol Chem*, 239:4081-4090, 1964.

Tottmar, S.O.C.; Pettersson, H.; and Kiessling, K.-H. The subcellular distribution and properties of aldehyde dehydrogeneses in rat liver. *J Biochem* (Tokyo), 135:577-586, 1973.

Tottmar, S.O.C.; Kiessling, K.H.; and Forsling, M. Effects of phenobarbital and ethanol on rat liver aldehyde dehydrogenases. *Acta Pharmacol Toxicol*, 35:270-276, 1974.

Tottmar, S.O.C.; Marchner, H.; and Pettersson, H. Determination of acetaldehyde in rat blood by the use of rat liver aldehyde dehydrogenase. *Ann Biochem*, 91:241-249, 1978.

Truitt, E.B. Blood acetaldehyde levels after alcohol consumption by alcoholics and nonalcoholic subjects. In: Roach, M.K.; McIsaac, W.M.; and Creaven, P.J., eds. *Biological Aspects of Alcohol*. Austin: University of Texas Press, 1971. pp. 212-232.

Truitt, E.G., and Duritz, G. The role of acetaldehyde in the actions of ethanol. In: Maickel, P.P., ed. *Biochemical Factors in Alcoholism*. New York: Pergamon Press, 1966. pp. 61-69.

von Wartburg, J.-P. Sensitivity to alcohol, a pharmacogenetic problem? In: *Proceedings of the International Medical Symposium on Alcohol and Drug Dependence*. Tokyo, Japan, 1977. p. 4.

von Wartburg, J.-P. Determination of acetaldehyde in human blood. *Experientia*, 35:1682-1683, 1979.

Walkenstein, S.S., and Weinhouse, S. Oxidation of aldehydes by mitochondria of rat tissues. *J Biol Chem*, 200:515-523, 1953.

Walsh, M.J. Role of acetaldehyde in the interactions of ethanol with neuroamines. In: Roach, M.K.; McIsaac, W.M.; and Creaven, P.J., eds. *Biological Aspects of Alcohol*. Austin: University of Texas Press, 1971. p. 233.

Weiner, H.; King, P.; Hu, J.H.J.; and Bensch, W.R. Mechanistic and enzymatic properties of liver aldehyde dehydrogenases. In: Thurman, R.G.; Yonetani, T.; Williamson, L.R.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems*. New York: Academic Press, 1974. pp. 101-125.

Wolff, P.H. Vasomotor sensitivity to alcohol in diverse mongoloid populations. *Am J Hum Genet*, 25:193-199, 1973.

Zeiner, A.R.; Paredes, A.; and Christensen, H.D. The role of acetaldehyde in mediating reactivity to an acute dose of ethanol among different racial groups. *Alcohol Clin Exp Res*, 3:11-18, 1979.

Brain Acetaldehyde Metabolism During Ethanol Consumption*

Dennis R. Petersen, V. Gene Erwin, and Richard A. Deitrich

Introduction

Several analytical methods have been developed to quantitate acetaldehyde in various biological samples (see Eriksson [1980] for a comprehensive review). The biological samples most commonly subjected to acetaldehyde determination are blood, breath, and cerebrospinal fluid. In many studies, this measurement has been conducted for the purpose of establishing physiological and pharmacological dose–response relationships for acetaldehyde in various peripheral tissues and in the central nervous system (CNS). The potential dose–response relationships for acetaldehyde in the CNS are of extreme importance, given the ability of this agent to form condensation products with various biogenic amines (Deitrich and Erwin 1980), to inhibit the oxidative metabolism of 5-hydroxyindole-acetaldehyde (Lahti and Majchrowicz 1969), and to interfere with the oxidation of 3,4-dihydroxyphenylglycolaldehyde, which in turn is inhibitory to $Na^+ + K^+$ - ATPase in brain (Deitrich and Erwin 1975). Despite the potential toxic actions of acetaldehyde described above, very few conclusive studies have appeared in the literature indicating that pharmacologically effective concentrations of acetaldehyde appear in the brain following ethanol ingestion. This paper is an attempt to survey the existing literature dealing with brain acetaldehyde levels following ethanol administration and to speculate which factors might be important in

---

*This work was supported in part by NIAAA grant AA03527.

controlling concentrations of this toxic and reactive molecule in the CNS.

Acetaldehyde Concentrations in Brain

Acetaldehyde oxidation in brain is thought to be mediated by aldehyde dehydrogenase (EC 1.2, 1.3). This enzyme has been partially purified from brains of the pig (Duncan and Tipton 1971), rat (Erwin and Deitrich 1966), and mouse (Petersen and Atkinson in press). Results of these studies indicate that aldehyde dehydrogenase (ALDH) present in brain of these species exhibits extremely low K_m values (0.1 μM to 2.0 μM) for acetaldehyde and a number of other aliphatic, aromatic, and biogenic aldehydes. Data from the above studies concerning subcellular localization of ALDH verify its presence in cytosolic and mitochondrial fractions of both rat and mouse brain. In rats and mice, approximately 40 percent of the total ALDH activity is present in the cytosolic fraction, whereas 50 to 60 percent is of mitochondrial origin. Petersen and Atkinson (in press) have demonstrated that in mouse brain, one ALDH enzyme present in the mitochondrial has a low K_m value (0.5 μM to 1.0 μM) for acetaldehyde, whereas other ALDH isozymes in the cytosolic compartment have K_m values for the same substrate on the order of 50 μM to 100 μM. Since blood acetaldehyde concentrations seldom exceed 50 μM in the laboratory mouse following ethanol administration, it appears that the low K_m mitochondrial ALDH is responsible for the majority of acetaldehyde oxidation in mouse brain.

On the basis of kinetic data obtained in the above studies, it appears that ALDH present in brain could be functioning at maximal velocity in an environment containing acetaldehyde in concentrations ranging from 5 μM to 10 μM. Thus, specific ALDH enzymes have the kinetic capabilities of maintaining extremely low levels of acetaldehyde in the brain during ethanol intoxication.

A limited number of studies have been conducted to obtain estimates of the in vivo capacity of rat and mouse brain to oxidize acetaldehyde. In most of these studies, brain acetaldehyde was measured after rapid removal of the brain at specific intervals following administration of ethanol alone or with acetaldehyde. Using methods that partially correct for the spontaneous formation of acetaldehyde in tissue and blood, Sippel (1974) measured blood and brain acetaldehyde levels in the rat following ethanol administration. This investigator reported that at blood acetaldehyde levels typically occurring during ethanol intoxication in the rat (10 μM to 100 μM), brain acetaldehyde levels were very low (15 μM) or not detectable. However, as blood acetaldehyde levels approached 200 μM, significant concentrations of acetaldehyde were detectable in brain. Eriksson and Sippel (1977) have confirmed that these results suggest the capillaries in brain are probably the rate-limiting metabolic barrier. As blood acetaldehyde levels approach 200 μM, the capacity of this metabolic barrier is exceeded.

Studies by Tabakoff et al. (1976) indicated that a similar relationship exists between blood and brain acetaldehyde concentrations in the laboratory mouse. These investigators administered ethanol and acetaldehyde in combination by the intraperitoneal route and found that until blood levels of acetaldehyde reached 200 μM, brain acetaldehyde levels were maintained at 10 μM. At blood acetaldehyde levels of 200 μM to 500 μM, brain concentrations ranged from 40 μM to 80 μM. However, brain acetaldehyde equilibrated with blood acetaldehyde as blood acetaldehyde levels approached 1 μM.

Petersen and Tabakoff (1979) used pargyline to specifically inhibit mouse liver ALDH in order to elevate blood acetaldehyde levels following ethanol administration and to avoid coadministering ethanol and acetaldehyde. Following parygline treatment, hepatic ALDH activity was inhibited 60 to 90 percent, whereas brain ALDH activity was not significantly decreased. Quantitation of brain acetaldehyde concentrations in 50 mice at various times following ethanol administration indicated that until blood acetaldehyde levels exceed 150 μM, brain acetaldehyde concentrations are maintained in the range of 5 μM to 20 μM. A polynomial equation fitted to these data suggests that in the laboratory mouse, brain concentrations of acetaldehyde would be near zero when blood acetaldehyde levels are 50 μM or below.

Results from these studies are consistent with respect to very low concentrations of acetaldehyde in brain cells following ethanol administration. However, several publications have appeared indicating that following ethanol ingestion, acetaldehyde can be detected in cerebrospinal fluid (CSF). Using the rat as an animal model, Petterssen and Kiessling (1977) correlated acetaldehyde concentration in tail blood, or blood obtained from the carotid artery, with acetaldehyde concentrations in CSF. As acetaldehyde levels exceeded 50 μM in blood obtained from the tail or carotid artery, CSF acetaldehyde concentrations began to increase, but were maintained below those occurring in blood. If the rats were pretreated with a diet containing cyanamide, a potent inhibitor of aldehyde dehydrogenase, acetaldehyde in CSF fluctuated as a direct function of blood acetaldehyde, indicating that brain ALDH may have been inhibited by cyanamide. These results implicate the importance of brain ALDH in regulating acetaldehyde levels in CSF. Kiianmaa and Virtanen (1978) conducted similar studies in rats using cerebroventriculo-cisternal perfusion techniques for continuous monitoring of acetaldehyde and ethanol in CSF during ethanol oxidation. From 60 to 240 minutes following ethanol administration, acetaldehyde concentrations in the CSF were threefold lower than those observed in blood. Although these rats were fed a diet contaminated with cyanamide, the results support the existence of a mechanism that limits the entry of acetaldehyde into the brain.

More recently, Stowell et al. (1980) measured blood, breath, and CSF acetaldehyde concentrations in intoxicated humans. Blood and breath levels of acetaldehyde were generally quite low (1 μM to 10 μM), whereas CSF levels were extremely low (1 μM to 5 μM). On the basis of these data, these investigators concluded that acetaldehyde concentrations in the brain may be too low for pharmacological importance.

Westcott et al. (1980) used a push-pull perfusion technique to measure acetaldehyde concentrations in interstitial fluid samples collected from the caudate nucleus and thalamus-hypothalamus region of rat brain. Concentrations of acetaldehyde in interstitial fluid were compared with those occurring in samples collected simultaneously from blood and whole brain. Following ethanol administration, blood acetaldehyde levels ranged from 5 μM to 50 μM. However, whole brain acetaldehyde levels were detectable only in rats pretreated with disulfiram, which exhibited blood acetaldehyde levels greater than 50 μM. These interesting results indicate that acetaldehyde can cross the blood-brain barrier and that it is not totally metabolized by the capillaries as suggested previously (Eriksson and Sippel 1977; Sippel 1974). In addition, since no acetaldehyde was detected in whole brain, the brain cells must metabolize acetaldehyde to a level not detectable by gas chromatography.

Tabakoff et al. (1976) have published calculations that describe the ability of mouse brain preparations to metabolize acetaldehyde. These investigators reported that in vitro preparations of mouse brain were capable of metabolizing 104 nmoles of acetaldehyde/min/g wet weight. Assuming a blood flow of 0.8 ml/min/gm brain and blood acetaldehyde levels of 60 μM, acetaldehyde would be delivered to the brain at a rate of 50 nmoles/min/gm brain. Thus, the mouse brain tissue can metabolize acetaldehyde at a faster rate than the rate at which acetaldehyde would be presented to the brain during normal blood flow. From the studies cited above, it appears that similar acetaldehyde metabolizing profiles may exist in rat and human brain.

Genetic and Environmental Factors Affecting Brain Acetaldehyde Levels

At present, there have been no published studies that indicate that genetic factors influence brain acetaldehyde levels in humans. However, data have appeared in the literature indicating that following an acute dose of ethanol, brain acetaldehyde levels are nearly twofold higher in DBA/2 mice as compared with C57BL/6 mice (Tabakoff et al. 1977). These results were explained on the basis of divergent strain differences in the K_m value of mouse brain ALDH for acetaldehyde. In the study, the K_m of brain ALDH from DBA/2 mice was reported to be 425 μM, whereas C57BL/6 mouse brain ALDH exhibited a K_m value of 93 μM. It is interesting to note that when challenged with a 3 g/kg dose of ethanol, C57BL/6 mice chronically treated with alcohol were found to have significantly higher brain acetaldehyde levels (twofold) as compared with isocaloric controls. However, DBA mice treated identically did not show this effect. Thus, it appears that in the laboratory mouse, genetic differences in brain acetaldehyde oxidizing capacity may be manifested at two levels. First, a genetic variation may exist because of kinetic differences in the enzymatic mechanisms responsible for acetaldehyde oxidation. Second, the ability of brain acetaldehyde oxidizing mechanisms might be differentially damaged by chronic alcohol consumption. The fact that chronic alcohol consumption significantly decreases acetaldehyde oxidation by hepatic mitochondria was reported by Hasumura et al. (1975). The effect of chronic alcohol consumption on mitochondrial acetaldehyde oxidation was attributed to a failure of the mechanisms responsible for generating reducing equivalents or altered rates of NADH reoxidation. It is possible that similar toxicities might be manifested by brain mitochondria in animals or humans chronically ingesting alcohol. However, additional studies are warranted to characterize the effect of chronic ethanol ingestion on similar brain mitochondrial functions and to determine if any genetic predispositions underlie such toxic manifestations.

From the data presented above, it appears that following ethanol ingestion, acetaldehyde levels in brain cells are in the range of 1 μM to 5 μM. However, even low concentrations of acetaldehyde may have a profound effect on biogenic amine and aldehyde metabolism. Since the K_m value of acetaldehyde in brain mitochondrial preparations is of the order of 1 μM (Erwin and Deitrich 1966; Petersen and Atkinson in press), it may be possible that biogenic aldehyde metabolism could be significantly inhibited by very low concentrations of acetaldehyde. Accumulations of the various biogenic aldehydes may result in a more profound effect than acetaldehyde itself. Under these conditions, acetaldehyde would be involved only indirectly, but could exert an "amplifying effect" on the actions of various biogenic aldehydes, as proposed by Deitrich and Erwin (1975).

Conclusion

The questions concerning the action of acetaldehyde in the central nervous system are important with respect to the acute and chronic effects of alcohol. These questions become even more complex when one considers the possibility of individual variation in response to the toxic actions of this very reactive molecule. Undoubtedly, the use of genetic principles and individual variation as tools in studying the acute and chronic actions of acetaldehyde will prove beneficial.

References

Deitrich, R.A., and Erwin, V.G. Involvement of biogenic amine metabolism in ethanol addiction. *Fed Proc*, 3(10):1962-1968, 1975.

Deitrich, R.A., and Erwin, V.G. Biogenic amine-aldehyde condensation products: Tetrahydroisoquinolines and tryptolines (β-carbolines). *Ann Rev Pharmacol Toxicol*, 20:55-80, 1980.

Duncan, R.J.S., and Tipton, K.F. The kinetics of pig brain aldehyde dehydrogenase. *Eur J Biochem*, 22:538-543, 1971.

Eriksson, C.J.P. Problems and pitfalls in acetaldehyde determinations. *Alcohol Clin Exp Res*, 4(1):22-29, 1980.

Eriksson, C.J.P., and Sippel, H.W. The distribution and metabolism of acetaldehyde in rats during ethanol oxidation. I. The distribution of acetaldehyde in liver, brain, blood and breath. *Biochem Pharmacol*, 26:241-247, 1977.

Erwin, V.G., and Deitrich, R.A. Brain aldehyde dehydrogenase: Localization, purification and properties. *J Biol Chem*, 241:3533-3539, 1966.

Hasumura, Y.; Teschke, R.; and Lieber, C.S. Acetaldehyde oxidation by hepatic mitochondria: Decrease after chronic ethanol consumption. *Science*, 189:127, 1975.

Kiianmaa, K., and Virtanen, P. Ethanol and acetaldehyde levels in cerebrospinal fluid during ethanol oxidation in the rat. *Neurosci Lett*, 10:181-186, 1978.

Lahti, R.A., and Majchrowicz, E. Acetaldehyde: An inhibition of the enzymatic oxidation of 5-hydroxyindoleacetaldehyde. *Biochem Pharmacol*, 18:535, 1969.

Petersen, D.R., and Atkinson, N. The purification and properties of mouse brain aldehyde dehydrogenase. In: Usdin, E.; Weiner, N.; and Youdim, M.B.H., eds. *Structure and Function of Monoamine Enzymes*. New York: Dekker, in press.

Petersen, D.R., and Tabakoff, B. Characterization of brain acetaldehyde oxidizing systems in the mouse. *Drug Alcohol Depend*, 4:137-144, 1979.

Petterssen, H., and Kiessling, K.-H. Acetaldehyde occurrence in cerebrospinal fluid during ethanol oxidation in rats and its dependence on blood level and dietary factors. *Biochem Pharmacol*, 26:237-240, 1977.

Sippel, H.W. The acetaldehyde content in rat brain during ethanol metabolism. *J Neurochem*, 23:451-452, 1974.

Stowell, A.; Hillbom, M.; Salaspuro, M.; and Lindros, K.O. Low acetaldehyde levels in blood, breath and cerebrospinal fluid of intoxicated humans assayed by improved methods. In: Thurman, R.G., ed. *Alcohol and Aldehyde Metabolizing Systems. IV. Advances in Experimental Medicine and Biology*. Vol. 132. New York: Plenum Press, 1980. pp. 635-645.

Tabakoff, B.; Anderson, R.A.; and Ritzmann, R.F. Brain acetaldehyde after ethanol administration. *Biochem Pharmacol*, 25:1305-1309, 1976.

Tabakoff, B.; Anderson, R.A.; and Ritzmann, R.F. Ethanol and acetaldehyde metabolism during ethanol consumption. In: Thurman, R.; Williamson, J.; Drott, H.; and Chance, B., eds. *Alcohol and Aldehyde Metabolizing Systems*. Vol. III. New York: Academic Press, 1977. pp. 555-565.

Westcott, J.Y.; Weiner, H.; Shultz, J.; and Myers, R.D. In vivo acetaldehyde in the brain of rat treated with ethanol. *Biochem Pharmacol*, 29(3):411-417, 1980.

Assessment of Platelet Monoamine Oxidase Activity As a Risk Factor in Epidemiologic Research*

Edward G. Shaskan

Introduction

Low platelet MAO activity among cross-cultural cohorts of patients with chronic undifferentiated schizophrenia is reported to be the most replicated neurochemical finding in schizophrenia (Buchsbaum et al. 1980). A similar replication, perhaps more robust, is the overwhelming incidence of low MAO individuals among populations diagnosed with another chronic psychiatric disease, alcoholism. Statistically significant differences between chronically ill and control populations' mean values for platelet MAO activity exist, despite widely different laboratory methods for platelet preparation and enzyme determination (table 1; Jackman and Meltzer 1980; Wise et al. 1980; Zeller and Davis 1980). However, failures to replicate the low-MAO phenomenon may be ascribed to issues of diagnosis and biologic heterogeneity (Buchsbaum and Rieder 1979; Buchsbaum et al. 1980).

Selection bias in retrospective case control studies of platelet MAO activity is reported even for control populations (Berger et al. 1978; Major and Murphy 1978). Therefore, it is likely that evaluation of low platelet MAO activity as a putative risk factor for alcoholism depends on complex issues of population selection.

Note: Figure and tables appear at end of paper.

*This work has been supported by the Alcohol Research Center, NIAAA grant P50AA03510, and U.S. Public Health Service grant AI15901.

Inherent problems in case control studies of alcoholism are further underscored by potential acute and chronic effects of this drug and its metabolism.

This paper reviews the literature on platelet MAO activity and alcoholism and focuses on state versus trait issues. Some results from multiple evaluations of platelet MAO activity in patients at our Alcohol Research Center are presented. Concepts of platelet MAO activity as a risk factor are discussed in light of established principles of epidemiology.

Table 1 presents a summary of seven published studies on platelet MAO activity and alcoholism. Historical progression of these studies is from clinical case–control approaches (studies 1 through 4) to population and family surveys (studies 5 through 7). Despite differences in study design, in each of these reports there is evidence for lower mean platelet MAO activity among populations diagnosed with alcoholism as compared with controls (table 1).

In some instances, low MAO may exist in subsets of patients; this finding may reflect diagnostic heterogeneity. For example, Takahashi and coworkers (1976) reported mean low platelet MAO activity among patients admitted with delirium tremens (DTs). However, no significant differences existed for mean MAO between patients without DTs and controls. Another subset (perhaps eight) of patients with DTs was studied longitudinally over 4 weeks and led the authors to conclude that low platelet MAO was state dependent (Takahashi et al. 1976). Similarly, selection bias of a subset of subjects could exist in the study by Brown (1977) in which significantly lower MAO was evident for six alcoholics abstinent for up to 5 months. In contrast, more consistent MAO values were found for eight case–control pairs whose abstinence exceeded 1 year (Brown 1977).

Originating from studies on the brain enzyme, and particularly on MAO evaluations in brains from alcoholic suicides, Swedish workers (Wiberg et al. 1977) published an extensive case–control study using two substrates (PEA and tryptamine) and assessing platelet MAO activity in alcoholics, serially with abstinence. This study concluded that low platelet MAO among the alcoholic population was not due to iron deficiency nor to serum acetaldehyde levels, and the authors suggested that low MAO is a biological factor predisposing to alcohol abuse.

Sullivan and coworkers (1978) provided the first long-term (up to 1 year) followup of interviewer-ensured abstinent alcoholics. Using well-defined criteria for alcoholism, these workers concluded that the low MAO in alcoholics, as compared with age-matched controls, represented evidence for a stable risk factor. One logical extension of this stable risk factor concept was to evaluate incidence of alcoholism and platelet MAO activity among first degree relatives of index alcoholic probands. Thus, Sullivan et al. (1979) could demonstrate a fourfold higher incidence of psychiatric hospitalization, including alcoholism, among first degree relatives of low MAO probands as compared with high MAO probands. First degree relatives of the lowest decile cohort of index alcoholics also had a significantly lower mean platelet MAO activity than controls or relatives with no history of psychiatric illness (Sullivan et al. 1979). These results are consistent with highly significant correlations between platelet MAO activity in normal twins (Nies et al. 1973; Winter et al. 1978), among well relatives of bipolar depressed patients (Leckman et al. 1977), and in well twins of schizophrenics in a monozygotic twin study (Wyatt, Murphy, Belmaker, Cohen, Donnelly, and Pollin 1973). Thus, in the light of this other evidence, the studies of Sullivan et al. (1978, 1979) lend strong support to the notion that MAO is a genetic risk factor in alcoholism.

Major and Murphy (1978) also reported a high incidence of psychiatric disease among first degree relatives of alcoholics with low platelet MAO. Their inclusion of military and nonmilitary control populations with lower platelet MAO activity distributions in the former populations could imply a nonspecific role for this purported vulnerability factor, and disease outcomes may result from algebraic summations with other biologic and psychosocial risk factors. Neither direct nor indirect effects of alcohol were responsible for low platelet MAO activity observed in the alcoholic group as deduced from regression analyses of platelet MAO activity on severity and chronicity of drinking and in the absence of any correlation with time of last drink (Major and Murphy 1978).

Although alcoholism was not a direct departure for their study, the results of Puchall et al. (1980) deserve scrutiny primarily because the low MAO probands were college students who were not alcoholics. Significantly lower MAO levels were found for the parents of low platelet MAO probands than for the parents of high MAO probands, and the incidence of alcoholism among the parents of low MAO probands was significantly greater ($p < 0.025$) than among parents of high MAO probands (Puchall et al. 1980). In agreement with the results of studies 3 through 6 (table 1), the observations of Puchall et al. (1980) make it unlikely that alcohol causes the low MAO activity frequently found among individuals with this diagnosis.

In summary, accounting for methodological and sampling factors in the first studies (table 1), a consensus of data reveals a low mean platelet MAO activity among distinct social and ethnic populations of alcoholics. However, definitive evidence for MAO activity as a biological factor at risk for alcoholism would require a fully prospective epidemiologic study. In the absence of time and resources for such a study, family studies and other methodologies tend to support the notion that low platelet MAO activity is a genetically determined trait predisposing to alcoholism. Whether this trait can be confounded by outcome factors in the pathogenesis of this disease is the subject of present ongoing research.

Methods

Alcoholic subjects (Feighner et al. 1972) were recruited from the alcohol treatment program at the University of Connecticut. Subjects were males and females ranging in age from 19 to 61 years. For comparison purposes, mean platelet MAO activity is presented for other populations studied concurrently within this laboratory. For all populations of subjects, informed consent was obtained according to the regulations of the University of Connecticut.

Bloods were collected in acid-citrate-dextrose (ACD-A) Vacutainers, and platelet pellets were prepared according to the method of Wyatt, Saavedra, and Axelrod (1973) with some modifications (Shaskan and Becker 1975). Briefly, the sequential differential centrifugations (10 minutes each) of the Vacutainer, producing platelet-rich plasma (PRP), at each step were as follows: 170×G, 380×G, 680×G. Polypropylene tubes and disposable plastic pipet tips were used for all transfers of PRP. The combined PRP was centrifuged again at 380×G for 10 minutes to remove incidental contamination by red cells. This final PRP was transferred to a clean tube and centrifuged at 10,000×G for 10 minutes and the platelet-poor plasma discarded. The platelet pellet was then rinsed three times successively with approximately 4 ml each of normal saline, drained, and stored at -70°C.

Platelet pellets were sonicated in 0.1 ml of 0.05 M sodium-potassium phosphate (Na-K-PO_4) buffer, pH 7.2 per ml of final PRP using a Kontes Cell Disrupter with power setting at number three and frequency setting between numbers three and four (Shaskan and Becker 1975). Conditions for the radiometric assay followed those already described, and ^{14}C-tryptamine (New England Nuclear) was used as substrate at final concentrations of 17 μM and 100 μM. The latter concentration of substrate for the assay was determined to be saturating and optimal in alcoholic and nonalcoholic subjects. Triplicate aliquots of the platelet sonicate were assayed for protein content by the method of Lowry et al. (1951) using bovine serum albumin as standard. As described earlier (Shaskan and Becker 1975), radioactivity was determined in a scintillation spectrometer with efficiencies at approximately 90 percent. MAO activity was expressed as nanomoles of product formed per milligram protein per hour, using batch-calculated specific activities of ^{14}C-tryptamine for calculations.

Batch-prepared platelet pellet samples from the American Red Cross Blood Bank ("outdated" platelet concentrates) were included in every assay as a control for day-to-day variation. These samples also provided triplicate blank assay values, which, following the addition of pargyline (100 μM final concentration), were preincubated for 10 minutes before addition of the radioactive substrate. The latter procedure gave more consistent and lower blanks in comparison with the previous method of boiling at 100°C for 10 minutes (Shaskan and Becker 1975), and sample radioactivity was always at least three times the enzyme blank values.

Results

Since sex- and age-matched controls (i.e., case controls) were not available for the study population of alcoholics, samples of platelets from other study populations concurrently being evaluated in the laboratory are presented for comparative purposes (table 2). Mean platelet MAO activity for our sample of 43 alcoholic males is significantly lower ($p < 0.005$) than for 83 college-age males (table 2). Compared with affective disorder (DSM II 296-298), represented here by 18 hospitalized patients with approximately the same age distribution and chronicity of illness, mean platelet MAO activity in alcoholism is also significantly lower ($p < 0.05$; table 2).

Compared with reference control populations, statistically significant deficits in mean platelet MAO activity were observed for male patients diagnosed with alcoholism ($p < 0.005$; table 3) and for females diagnosed with alcoholism ($p < 0.001$; table 3). Lower mean platelet MAO activity existed for both populations of alcoholic patients than for controls. This finding was apparent whether platelet MAO activity was evaluated near the K_m value for tryptamine (17 μM) or at saturating levels of substrate (100 μM). At the 100 μM substrate concentrations, increased variation around the mean precluded statistical significance in a comparison of males and lowered the value for comparison of female populations to $p < 0.005$ (table 3).

A graphic relationship between platelet MAO activity in alcoholic males and normal reference males is shown in figure 1. Mean platelet MAO activity for each period following admission to hospital did not significantly vary over the sampling period during hospitalization, nor at 1 year, using either 17 μM or 100 μM tryptamine (figure 1). There was a trend toward increased mean platelet MAO activity at day 9 of hospitalization, but this figure is not statistically significant (figure 1).

Mean platelet MAO values for male and female alcoholic patients at 1-year followup were not significantly different from mean values for these populations during hospitalization. Conceivably, the observed mean values summate from opposing vectors of decreased MAO among individuals who drank during the year and increased MAO for those who abstained from alcoholic beverages. Thus, of those who completed a 1-year posthospitalization intensive followup surveillance, approximately one-half remained abstinent for this year (table 4). Partitioning followup subjects according to a median split of MAO activity (reference male and female equal 2.83 and 3.49, respectively) results in a distribution of "drinkers" within the low MAO quadrant ($p = 0.07$; table 4). Platelet MAO activity may increase with age (Robinson et al. 1977), but the distribution of drinkers and abstainers did not significantly differ with respect to age. Mean values of platelet MAO activity were lower for male and female "drinkers" than for "abstainers" (table 5), but these comparisons did not reach statistical significance at the $p = 0.05$ level.

Since ethanol can induce a thrombocytopenic state (Sahud 1972) and other physiological variations can result from at least acute ethanol ingestion, sequential platelet MAO activities were assessed in relation to patients who came into the hospital with either depressed platelet counts or depressed serum ethanol, or both (table 6). No obvious relationship between platelet count and/or serum ethanol levels with platelet MAO could be seen (table 6). There seemed to be more variability in platelet MAO activity among subjects who were admitted with either the lowest platelet counts or the highest serum ethanol levels (e.g., subjects 1, 2, and 3 of group A and subject 12 of group C, table 6). Relative stability of platelet MAO activity was observed in other subjects admitted with high serum ethanol levels (e.g., subjects 8 through 11 of group C, table 6). No consistent relationship was found between serum iron and/or total iron binding capacity to platelet MAO activity at time of admission to hospital.

Discussion

Superficially, the results of the present study support the consensus of data collected in table 1, which reveals consistently low mean platelet MAO activity among populations diagnosed with alcoholism. As reported in table 2, mean platelet MAO activity for 43 hospitalized male patients was significantly lower than for reference normals ($p<0.005$) and lower than for a population of approximately aged-matched hospitalized patients with affective disorder ($p<0.05$). Low MAO among this population of alcoholics also contrasts with the MAO of a small sample ($N = 7$) of patients diagnosed with antisocial personalities, but is comparable to mean values for patients diagnosed with schizophrenia (table 2). Although these results on patients with schizophrenia (many with early onset) support numerous reports in the literature (reviewed by Wyatt et al. 1979, 1980), possible chronic effects of antipsychotic medication in this population cannot be disregarded (Friedhoff et al. 1978; Friedhoff and Miller 1980). Therefore, mean and variation of platelet MAO in patients with alcoholism and schizophrenia, contrasted with MAO in college students (table 2), could represent a pharmacological outcome of ethanol and antipsychotics, respectively.

Other possible confounding effects, including laboratory methodology, are recognized. For example, Wise et al. (1980) and Jackman and Meltzer (1980) emphasized the importance of optimal substrate concentration and platelet preparation for accurate and reproducible evaluation of MAO activity. Principally because of large-scale epidemiologic studies in progress, this laboratory utilizes differential centrifugation and triplicate rinsing of the platelet pellet to remove plasma proteins. Using these methods, yearly comparisons of MAO activity in college studies yield Pearson's *r* correlation coefficients averaging 0.6 ($p<0.001$; Shaskan et al. 1980). Over shorter intervals of 1 to 2 months, this laboratory observes higher correlations of platelet MAO activity ($r>0.8$) for healthy adult males (Shaskan unpublished observations). This confirms previous reports of stability of platelet MAO over time (Murphy, Belmaker, Carpenter, and Wyatt 1977). Among younger and healthier male and female patients in the present study, coefficients of variation for platelet MAO activity over 3 weeks' in-hospital evaluation range from 5 to 18 percent. Weekly variations in this enzyme's activity in older and frequently sicker patients with alcoholism is often considerable (table 6). However, no consistent relationship between platelet counts and/or serum ethanol with MAO activity is apparent (table 6).

Since differential centrifugation and expressing platelet MAO activity per platelet protein is more suitable for epidemiologic studies, and since this is an established procedure in our laboratory, continuation of this method would seem to better serve the relevance of epidemiology rather than the "perfection" (on a membrane-bound enzyme?) of clinical chemistry. This opinion on a controversial issue relates to broader statistical and practical considerations, especially if platelet MAO activity is to be prospectively evaluated as a putative risk factor in epidemiologic (population) research.

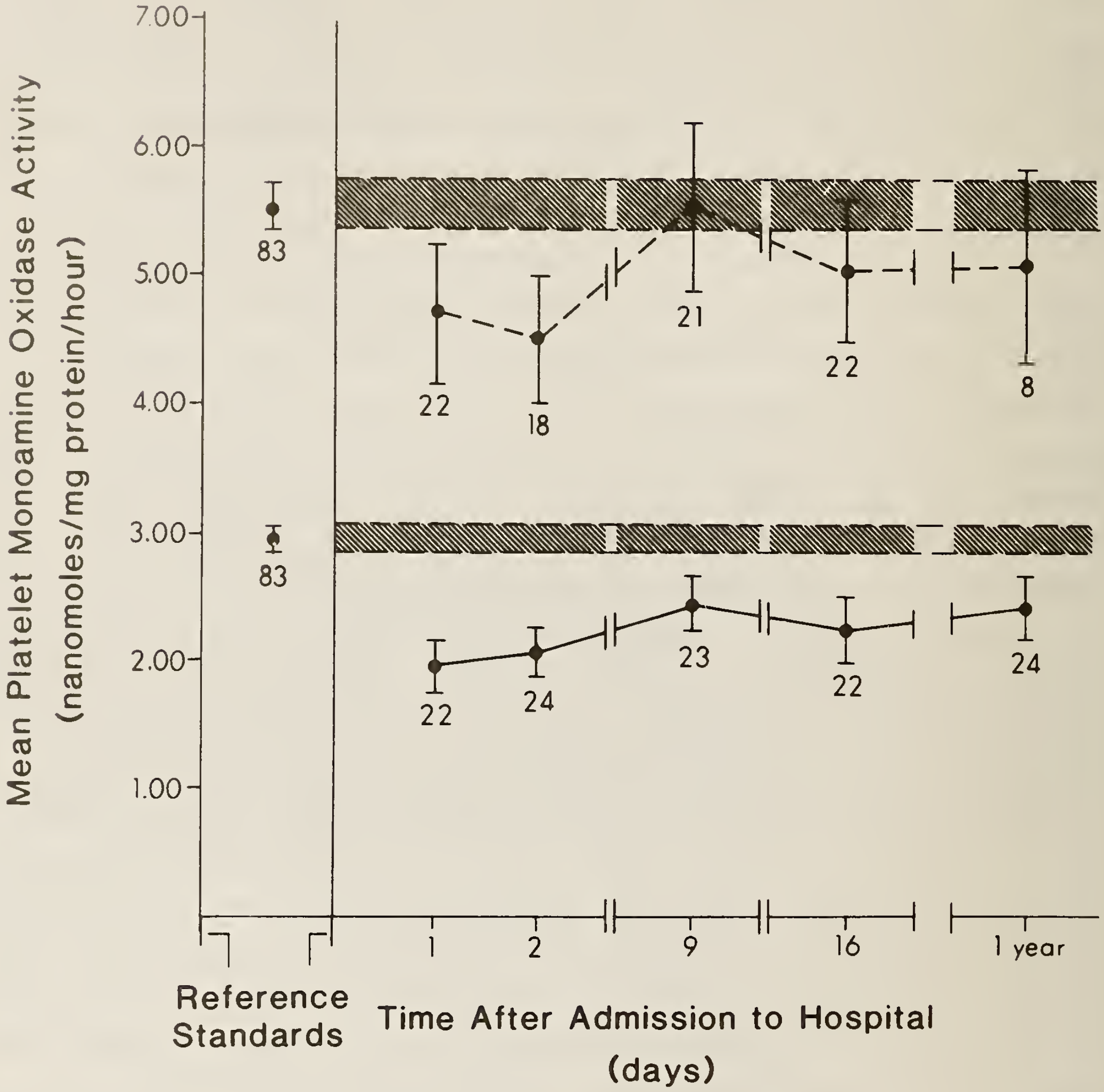
Regrettably, an established "error" in this laboratory's MAO assay (using suboptimal tryptamine concentrations) might have led to erroneous interpretations and comparisons between patient and control groups (Wise et al. 1980). Accordingly, evaluation of tryptamine concentration at 17 μM, 100 μM, and 200 μM was done on male and female patients with alcoholism and on controls, using the procedures outlined in "Methods," and 100 μM tryptamine was found to be saturating and in the optimal range for the assay. As shown in table 3 and figure 1, mean platelet MAO activity among patients with alcoholism is lower than among reference controls at 100 μM and 17 μM tryptamine concentration. However, more variability among the patient groups than among the reference controls, especially at 100 μM tryptamine (figure 1, table 3), reduces the statistical reliability of these comparisons.

Although mean platelet MAO activity at 17 μM and 100 μM tryptamine concentration did not significantly vary over sampling days in hospital (figure 1), the trend toward increased MAO levels after 9 days of abstinence with return to lower levels 1 week later is consistent with statistically significant fluctuations over a similar abstinent time period independently reported (Wiberg et al. 1977; Wiberg 1979). Although Wiberg (1979) discussed the possible contributions of adrenalin and the effect of stress on this transitory phenomenon, Takahashi et al. (1976) reported a somewhat different response of platelet MAO among their cohort of abstinent patients. Typological differences within and across cohorts of patients with alcoholism could explain these differences. The incidence of DTs, for instance, is higher in the Japanese study (Takahashi et al. 1976). In the present study, only two male patients represented in figure 1 required detoxification from DTs upon admission to hospital. In fact, approximately 60 percent of all patients in the present study entered the hospital with undetectable serum ethanol levels. Other typological issues, pathologic (e.g., liver disease, endocrinopathies) and psychologic (e.g., depression), can be addressed in future reports with larger samples.

A relevant typology prospectively generated is the followup data leading to abstinent versus drinking cohorts. Although these cohorts are still numerically small, a comparison of 1-year followup abstainers and drinkers suggested that long-term (greater than 10 months) alcohol abuse could lower platelet MAO activity (tables 4 and 5). However, since neither male nor female abstainers' mean and distribution of MAO activity return to reference values by the 1-year followup, low platelet MAO activity could also be a risk factor (predisposing and/or precipitating) for alcoholism. This evidence for confounded risk factors is not unique to alcoholism, but is common to all case–control studies of chronic disease.

Identification of necessary agents in infectious and chronic disease does not imply sufficient causality for pathogenesis of those diseases (Barrett-Connor 1979; Evans 1979). In addition to psychosocial factors that predispose humans to a lifestyle of alcohol and substance abuse, biological host factors may predispose to and/or precipitate alcoholism. "Focus on the disease alone or use of case–control (retrospective) studies are unlikely to yield important data in this regard" (Evans 1979, p. 380).

Figure 1. Mean Platelet MAO Activity Over Time for Patients Admitted to the Alcohol Treatment Program



Note: Lower and upper curves represent tryptamine at 17 μM and 100 μM, respectively. Bars are the standard errors with subject numbers. At this time, only 8 of 22 subjects evaluated at 100 μM tryptamine have returned to 1-year followup. No distinction has been made for abstainers and drinkers at 1-year followup. Reference standards are college students with mean age of 20.5 ± 0.1 SEM.

Table 1. Published Studies on Platelet MAO Activity in Alcoholism

	Study	Number and Types of Subjects		Substrate	Results	Comments
		Controls	Alcoholics			
1.	Takahashi, Tani, and Yamane (1976)	26 M 24 F Professionals (41 ± 11 yrs)	48 M 2 F (47 ± 10 yrs)	Serotonin	State-dependent decrease associated with DT.	No difference between alcoholics and controls at 4 weeks.
2.	Brown (1977)	14 Professionals (25–68) Age- and sex-matched	14 Abstinent, outpatient volunteers	Benzylamine	With prolonged abstinence (>6 mos) alcoholics' MAO is more similar to controls.	Not possible to examine state-trait issue.
3.	Wiberg, Gottfried, and Oreland (1977)	Professionals Age- and sex-matched	21 M 3 F	β-phenylethylamine, tryptamine	Reduced MAO among alcoholics up to 25 days of abstinence; not due to iron or acetaldehyde.	Low MAO may reflect a constitutional quality of brain. MAO a genetic marker?
4.	Sullivan, Stanfield, Schanberg, and Cavenar (1978)	20 M (42.9 ± 2.0 SE, yrs)	27 M (44.7 ± 2.4 SE) "Feighner" criteria and DSM II	Tryptamine	Lower MAO as compared to controls at baseline, 6 mos and 12 mos of interviewer-ensured abstinence.	MAO is a stable risk factor.

Table 1. Published Studies on Platelet MAO Activity in Alcoholism—Continued

	Number and Types of Subjects				
Study	Controls	Alcoholics	Substrate	Results	Comments
5. Major and Murphy (1978)	42 M (military) (33.6 ± 2.1 yrs) 42 M (nonmilitary) (31.6 ± 3.3 yrs)	99 M (military) DSM II (34.2 ± 1.9 yrs)	Benzylamine	Relationship between mean MAO activity in three populations studied is alcoholics < military controls < nonmilitary controls.	MAO plus behavioral correlates support genetic vulnerability hypothesis.
6. Sullivan, Cavenar, Maltbie, Lister, and Zung (1979)	100 M (45.2 ± 1.9 yrs) Elective surgical patients	200 M probands (42.1 ± 2.1 yrs) DSM II and family members	Tryptamine	Lower MAO in alcoholics and first degree relatives with positive psychiatric history.	Importance of family studies of probands; compelling evidence that MAO is a risk factor in alcoholism.
7. Puchall, Coursey, Buchsbaum, and Murphy (1980)	Parents of low MAO probands (N = 37) Parents of high MAO probands (N = 38)	Incidence of alcoholism = 7/37 (19 percent) Incidence of alcoholism = 1/38 (3 percent)	Benzylamine	Generally positive and significant correlation between parents' and childrens' MAO levels; highly unlikely that assortative mating for MAO exists.	While low MAO activity may be a vulnerability factor for alcoholism, this is probably only one source within a multifactorial biopsychosocial etiology of alcoholism.

Table 2. Platelet MAO Activity Among a Variety of Male Populations According to Psychiatric Diagnoses (DSM II) and Age

Population	N	Mean ± SE Age (years)	Mean ± SE MAO Activity (nmoles/mg prot/hr)
College students (reference normals)	83	20.5 ± 0.1	2.93 ± 0.11[a]
Alcoholism	43	39.3 ± 1.8	2.31 ± 0.16[a]
Schizophrenia	69	23.7 ± 0.8	2.55 ± 0.13
Paranoid	33	24.1 ± 1.3	2.65 ± 0.21
Latent	8	20.1 ± 2.1	2.28 ± 0.17
Schizoaffective	19	24.3 ± 1.1	2.62 ± 0.23
Chronic, Undiff.	9	24.4 ± 2.0	2.29 ± 0.46
Affective disorder (DSM II 296–298)	18	45.5 ± 4.4	2.98 ± 0.23[b]
Antisocial personality (DSM II 301–308)	7	17.3 ± 0.6	3.13 ± 0.29

Note: Platelet MAO evaluations were done in triplicate with a final tryptamine concentration of 17 μM.

[a] $t_{124} = 3.33, p < 0.005$.

[b] $t_{59} = 2.35, p < 0.05$.

Table 3. Platelet MAO Activity at Two Substrate Concentrations for Laboratory Reference and Alcoholic Populations

Population (Mean age ± SEM)	Mean ± SEM Platelet MAO Activity (N) (nanomoles/mg prot/hr) 17 μM	100 μM
Reference		
Males (20.5 ± 0.1)	2.93 ± 0.11 (83)[a]	5.53 ± 0.16 (83)[c]
Females (18.4 ± 0.1)	3.53 ± 0.19 (28)[b]	6.69 ± 0.32 (35)[d]
Alcoholic patients		
Males (39.3 ± 1.8)	2.31 ± 0.16 (43)[a]	4.94 ± 0.43 (22)[c]
Females (38.0 ± 2.9)	2.59 ± 0.15 (27)[b]	4.98 ± 0.35 (13)[d]

Note: Patients' mean values were calculated from at least three samples collected on days 1, 2, 9, and 16 of hospital-assured abstinence. Triplicate MAO assays were performed on each sample.

[a] $t_{124} = 3.33; p < 0.005$.

[b] $t_{53} = 3.81; p < 0.001$.

[c] $t_{103} = 1.53; p = 0.13$.

[d] $t_{46} = 3.03; p < 0.005$.

Table 4. Chi-Square Distribution of Alcoholic Subjects Completing 1-Year Followup, According to Drinking Surveillance and Repartition Around Median Split of Reference (Control) Population Platelet MAO

	Abstinent	Drinkers
Low MAO	9 (5 M + 4 F)	18 (11 M + 7 F)
High MAO	9 (5 M + 4 F)	4 (3 M + 1 F)

Note: $\chi^2 = 3.23$, 1 df, $p = 0.07$.

Table 5. Mean Platelet MAO Activity for Subpopulations of Alcoholic Patients According to Drinking History at 1-Year Followup

Subjects Completing 1-Year Followup	Mean ± SE Platelet MAO Activity (nanomoles/mg prot/hr) Abstainers (*N*)	Drinkers (*N*)	Comparison of Means (Student's t test)
Males			
Hospital Day 9	2.59 ± 0.44 (10)	2.30 ± 0.20 (14)	$t_{22} = 0.66$ $p = 0.52$
Followup at 1 year	2.84 ± 0.47 (10)	2.13 ± 0.26 (14)	$t_{22} = 1.44$ $p = 0.16$
Females			
Hospital Day 9	3.13 ± 0.31 (8)	2.42 ± 0.48 (8)	$t_{14} = 1.24$ $p = 0.23$
Followup at 1 year	3.13 ± 0.42 (8)	2.64 ± 0.25 (8)	$t_{14} = 0.99$ $p = 0.34$

Note: Triplicate MAO evaluations were performed on each sample at a final tryptamine concentration of 17 μM.

Table 6. Serial Platelet MAO Activity for Alcoholic Males and Females Having Low Platelet Count and/or Detectable Serum Ethanol Upon Admission

Subjects (Sex and age)			Day 1 Determinations[a]		Platelet MAO Activity (nmoles/mg prot/hr)					Comment[b] at 1 Year
			Platelet Count (cells/mm³)	Serum Ethanol (mg/dl)	Day 1	Day 2	Day 9	Day 16	1 Year	
Group A										
1.	F	47	23,000	239	2.47	1.02	0.63	0.68	2.44	II
2.	M	47	55,000	89	0.89	—	5.39	1.97	—	—
3.	F	50	110,000	331	2.77	2.61	4.64	1.97	1.07	I
4.	F	33	123,000	13	0.98	—	—	1.30	—	—
Group B										
5.	F	50	56,000	0	4.43	3.88	4.64	0.73	1.35	II
6.	F	61	107,000	0	—	3.79	3.80	0.72	3.76	II
Group C										
7.	M	27	262,000	6	2.30	2.30	2.10	2.73	—	—
8.	M	39	244,000	78	3.12	3.45	3.64	2.61	—	—
9.	M	53	256,000	86	3.13	3.38	3.80	2.85	—	—
10.	M	44	243,000	155	1.23	1.45	1.14	0.92	1.62	III
11.	M	30	158,000	178	4.80	3.12	2.94	2.76	—	—
12.	F	57	305,000	206	3.10	—	0.93	6.28	—	—

[a] Platelet count reference values are 140,000–350,000; for serum ethanol levels, 100 mg/dl is considered legally intoxicated.

[b] I = abstinent; II = partly abstinent; III = drinking more than 10 months of the year, sometimes heavily.

References

Barrett-Connor, E. Infectious and chronic disease epidemiology: Separate and unequal? *Am J Epidemiol*, 109:245-249, 1979.

Berger, P.A.; Ginsburg, R.A.; Barchas, J.D.; Murphy, D.L.; and Wyatt, R.J. Platelet monoamine oxidase in chronic schizophrenic patients. *Am J Psychiatry*, 135:95-99, 1978.

Brown, J.B. Platelet MAO and alcoholism. *Am J Psychiatry*, 134:206-207, 1977.

Buchsbaum, M.S., and Rieder, R.O. Biologic heterogeneity and psychiatry research—Platelet MAO activity as a case study. *Arch Gen Psychiatry*, 36:1163-1169, 1979.

Buchsbaum, M.S.; Coursey, R.D.; and Murphy, D.L. Schizophrenia and platelet monoamine oxidase: Research strategies. *Schizophr Bull*, 6:375-384, 1980.

Evans, A. Definitions of epidemiology. *Am J Epidemiol*, 109:379-381, 1979.

Feighner, J.P.; Robins, E.; Guze, S.B.; Woodruff, R.A.; Winokur, G.; and Munoz, R. Diagnostic criteria for use in psychiatric research. *Arch Gen Psychiatry*, 26:57-63, 1972.

Friedhoff, A.J., and Miller, J.C. Platelet monoamine oxidase as a function of nongenetic factors. *Schizophr Bull*, 6:314-319, 1980.

Friedhoff, A.J.; Miller, J.C.; and Weisenfreund, J. Human platelet MAO in drug-free and medicated schizophrenic patients. *Am J Psychiatry*, 135:952-955, 1978.

Jackman, H.L., and Meltzer, H.Y. Factors affecting determination of platelet monoamine oxidase activity. *Schizophr Bull*, 6:259-266, 1980.

Leckman, J.F.; Gershon, E.S.; Nichols, A.S.; and Murphy, D.L. Reduced monoamine oxidase activity in first degree relatives of bipolar affective disorder—A preliminary report. *Arch Gen Psychiatry*, 34:601-606, 1977.

Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; and Randall, R.J. Protein measurement with the folin phenol reagent. *J Biol Chem*, 193:265-275, 1951.

Major, L.F., and Murphy, D.L. Platelet and plasma amine oxidase activity in alcoholic individuals. *Br J Psychiatry*, 132:548-554, 1978.

Murphy, D.L.; Belmaker, R.; Carpenter, W.; and Wyatt, R.J. Monoamine oxidase in chronic schizophrenia: Studies of hormonal and other factors affecting enzyme activity. *Br J Psychiatry*, 130:151-158, 1977.

Murphy, D.L.; Belmaker, R.H.; Buchsbaum, M.S.; Martin, N.F.; Ciranello, R.; and Wyatt, R.J. Biogenic amine-related enzymes and personality variations in normals. *Psychol Med*, 7:149-157, 1977.

Nies, A.; Robinson, D.; Lamborn, K.; and Lampert, R. Genetic control of platelet and plasma monoamine oxidase activity. *Arch Gen Psychiatry*, 28:834-838, 1973.

Puchall, L.B.; Coursey, R.D.; Buchsbaum, M.S.; and Murphy, D.L. Parents of high risk subjects defined by levels of monoamine oxidase activity. *Schizophr Bull*, 6:338-346, 1980.

Robinson, D.S.; Sourkes, T.L.; Nies, A.; Harris, L.S.; Spector, S.; Bartlett, D.L.; and Kaye, I.S. Monoamine metabolism in human brain. *Arch Gen Psychiatry*, 34:89-92, 1977.

Sahud, M.A. Platelet size and number in alcoholic thrombocytopenia. *N Engl J Med*, 286:355-356, 1972.

Shaskan, E.G., and Becker, R.E. Blood platelet monoamine oxidase activity in anergic schizophrenics. In: National Institute on Drug Abuse. *Aminergic*

Hypotheses of Behavior: Reality or Cliche? Research Monograph 3. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1975. pp. 29-35.

Shaskan, E.G.; Peszke, M.A.; and Niederman, J.C. Studies of platelet monoamine oxidase activity in Epstein-Barr and Dengue virus infections. *Schizophr Bull*, 6:347-354, 1980.

Sullivan, J.L.; Stanfield, C.N.; Schanberg, S.; and Cavenar, J.O., Jr. Platelet monoamine oxidase and serum dopamine-β-hydroxylase activity in chronic alcoholics. *Arch Gen Psychiatry*, 35:1209-1212, 1978.

Sullivan, J.L.; Cavenar, J.O.; Maltbie, A.A.; Lister, P.; and Zung, W.W.K. Familial biochemical and clinical correlates of alcoholics with low platelet monoamine oxidase activity. *Biol Psychiatry*, 14:385-394, 1979.

Takahashi, S.; Tani, N.; and Yamane, H. Monoamine oxidase activity in blood platelets in alcoholism. *Folia Psychiatr Neurol Jpn*, 30:455-462, 1976.

Wiberg, A. Increase in platelet monoamine oxidase activity during controlled abstinence after alcohol abuse. *Med Biol*, 57:133-134, 1979.

Wiberg, A.; Gottfries, C.-G.; and Oreland, L. Low platelet monoamine oxidase activity in human alcoholics. *Med Biol*, 55:181-186, 1977.

Winter, H.; Herschel, M.; Propping, P.; Friedl, W.; and Vogel, F. A twin study on three enzymes (DBH, COMT, MAO) of catecholamine metabolism: Correlations with MMPI. *Psychopharmacology*, 57:63-69, 1978.

Wise, C.D.; Potkin, S.G.; Bridge, T.P.; Phelps, B.H.; Cannon-Spoor, H.E.; and Wyatt, R.J. Sources of error in the determination of platelet monoamine oxidase: A review of methods. *Schizophr Bull*, 6:245-253, 1980.

Wyatt, R.J.; Murphy, D.L.; Belmaker, R.; Cohen, S.; Donnelly, C.H.; and Pollin, W. Reduced monoamine oxidase activity in platelets: A possible genetic marker for vulnerability to schizophrenia. *Science*, 179:916-918, 1973.

Wyatt, R.J.; Saavedra, J.M.; and Axelrod, J. A dimethyltryptamine-forming enzyme in human blood. *Am J Psychiatry*, 130:754-760, 1973.

Wyatt, R.J.; Potkin, S.G.; and Murphy, D.L. Platelet monoamine oxidase activity in schizophrenia: A review of the data. *Am J Psychiatry*, 136:377-385, 1979.

Wyatt, R.J.; Potkin, S.G.; Bridge, T.P.; Phelps, B.H.; and Wise, C.D. Monoamine oxidase in schizophrenia: An overview. *Schizophr Bull*, 6:199-207, 1980.

Zeller, E.A., and Davis, J.M. Protein content and monoamine oxidase activity in platelets. *Schizophr Bull*, 6:267-274, 1980.

Why Do Alcoholics Have Low Platelet Monoamine Oxidase Activity?

Lars Oreland

Localization and Characteristics of MAO in Human Platelets and Brain

The activity of monoamine oxidase (MAO, monoamine O_2: oxidoreductase, E C 1.4.3.4.) can be divided into two forms, MAO-A and MAO-B, where the A-form is sensitive to inhibition by the acetylenic inhibitor clorgyline and the B-form is sensitive to inhibition by 1-deprenil (Johnston 1968; Knoll and Magyar 1972). According to this definition, only the B-form of the enzyme is present in human platelets (Donnelly and Murphy 1977). Furthermore, it has been shown that human platelet MAO-B has a single binding site for monoamine substrates (Fowler and Wiberg 1980; Fowler, Ekstedt, Egashira, Kinemuch, and Oreland 1979). In the human brain, however, the oxidation of 5-hydroxytryptamine is brought about by MAO-A alone and the oxidation of low concentrations of β-phenylethylamine by MAO-B alone (Roth 1976; White and Glassman 1977). This finding seems to be true also for rat brain homogenates (Johnston 1968; Yang and Neff 1973). In the rat brain, the activity of MAO-A is found predominantly in the neurons, whereas the activity of MAO-B is localized in the main to the nonneuronal tissue (Student and Edwards 1977). There is good evidence that both forms of human brain MAO are homogenous (Fowler, Oreland, Markusson, and Winblad 1980; Fowler, Wiberg, Oreland, and Winblad 1980). The kinetic properties of human platelet and brain MAO-B appear to be rather similar, but not identical. In a recent review, the properties of human platelet and brain MAO-B were compared (Oreland, Wiberg, and Fowler in press), and it was suggested that the differences in the properties of the MAO-B from the two sources may be due to different lipid microenvironments.

Regulation of Human Platelet and Brain MAO

Human platelet MAO activity appears to be under strong genetic control as revealed by twin studies (Murphy 1976; Nies et al. 1973; Pandey et al. 1979; Winter et al. 1978). This genetic control seems to be exerted through a variation in enzyme concentration rather than a variation in K_m or molecular turnover number for the enzyme (Fowler and Wiberg 1980; Oreland 1980). There seems to be a remarkable stability of the enzyme activity with a high test-retest reliability over long time periods, and the influence of age, if present at all, is negligible (Murphy et al. 1976). Certain drugs, hormones, and stressful situations have, however, been found to change the activity (for a recent review, see Oreland, Wiberg, and Fowler in press).

There is some evidence that human brain MAO activity is under some form of genetic control. Thus, MacPike and Meier (1976) found that the brain MAO activity in young mice was determined mainly by the genotype. In the human brain, MAO-A and MAO-B activities are highly intercorrelated within the same region, and a correlation has also been found between the activity of either MAO-A or MAO-B in one brain region and the activity of either enzyme form in another brain region. This was true even when the samples had been corrected for the effects of age (Fowler, Wiberg, Oreland, Marcusson, and Winblad 1980). These results are consistent with the hypothesis that, in analogy with the mouse brain, there is some sort of genetic regulation of human brain MAO. The mechanism underlying the variation in brain MAO activity between different individuals seems to be the same as for the platelet enzyme; thus, the enzyme concentration rather than K_m or molecular turnover number was found to vary between individuals (Fowler, Wiberg, Oreland, Marcusson, and Winblad 1980; Fowler, Wiberg, Oreland, and Winblad 1980).

Human Platelet MAO Activity in Alcoholics

A low activity of human platelet MAO has been found to be associated with alcohol abuse (Agarwal et al. 1979; Major and Murphy 1978; Sullivan et al. 1978; von Knorring and Oreland 1978; Wiberg, Gottfries, and Oreland 1977), and in contrast to the situation for schizophrenia (see Wyatt et al. 1980), there seems to be no report of a normal mean activity in a group of alcoholics. This lower activity was not due to a direct effect of alcohol (Wiberg, Gottfries, and Oreland 1977), to differences in the iron content of the two groups (Major and Murphy 1978), nor to a direct effect of acetaldehyde on the MAO (Wiberg, Gottfries, and Oreland 1977) or to an effect secondary to the liver damage produced by alcohol abuse (Fowler, Wiberg, Oreland, Danielson, Palm, and Winblad in press). Furthermore, the low platelet MAO activity was not correlated to the severity or duration of the abuse (Major and Murphy 1978). In addition, plasma taken from alcoholics does not contain MAO-inhibitory factors (Oreland, Fowler, and Shalling unpublished results), which might have been a possibility when considering the report by Yu and Boulton (1979).

During the abstinence phase after cessation of alcohol abuse, there is a temporary increase in the activity (Fowler, Wiberg, Oreland, Danielson, Palm, and Winblad in press; Wiberg 1979; Wiberg, Gottfries and Oreland 1977) with a high test-retest reliability being found for the samples (Fowler, Wiberg, Oreland, Danielson, Palm, and Winblad in press). This transitory increase in the platelet MAO activity might be related to the increase in adrenergic activity that has been found to take place in the abstinence after ethanol abuse (Athen et al. 1977), especially as subcutaneous injections of adrenaline have been found to exert a temporary increase in platelet MAO activity (Owen et al. 1977). In some of the studies cited above, platelet MAO activity has been expressed as quantity of amine oxidized per unit time per unit platelet concentration, which would minimize the influence of the platelet concentration on the result. This possibility has, however, been thoroughly dealt with in a recent paper from our group (Fowler, Wiberg, Oreland, Palm, and Winblad in press), with the conclusion that the low platelet concentration found in alcoholics (Major and Murphy 1978; Fowler, Wiberg, Oreland, Danielson, Palm, and Winblad in press) does not explain the low platelet MAO activity found in alcoholics. The lower activity found in alcoholics is due entirely to a difference in true V_{max} value without change in the K_m value of the enzyme toward the substrate tested (tryptamine) (Fowler, Wiberg, Oreland, Danielson, Palm, and Winblad in press). No difference in the molecular turnover number of MAO in platelets from a low-MAO proband with a previous history of alcohol abuse was found with respect to that from a healthy medium-MAO proband (Oreland 1980).

Human Brain MAO Activity in Alcoholics

In a recent investigation, we found lower MAO-A activity in the brains of alcoholics in hypothalamus and caudatus but not in cortex gyrus cinguili or hippocampus (Oreland et al. submitted for publication). The activity of MAO-B also appeared to be lower in alcoholics than in controls, but this result should be interpreted with caution in view of the different ages of the controls and alcoholics. Thus, the activity of MAO-B but not MAO-A among controls was correlated positively with age both in this investigation and in previous ones (Eckert et al. 1980; Fowler, Wiberg, Oreland, Marcusson, and Winblad 1980), which is consistent with the hypothesis that there is a neuron less with age (Fowler, Wiberg, Oreland, Marcusson, and Winblad 1980); Oreland 1979). Indeed, if the MAO-B activities were compensated for age, the MAO-B activities would be higher for the alcoholics (Carlsson et al. 1980), consistent with the hypothesis that there is some brain damage in alcoholism (see Bergman et al. 1980), since a decrease in the neuronal:non-neuronal ratio produces an increase in the MAO-B activity. Such an increase in the activity of MAO-B but not in MAO-A has also been found in hemitransected rats (Fowler, Oreland, Wiberg, Carlsson, and Magnusson. 1979; Oreland et al. 1980) and in patients with Alzheimer's disease (Adolfsson et al. 1980).

Rat Brain MAO Activity in Relation to Ethanol

Chronic treatment of rats with ethanol, either by addition of ethanol to the drinking water for 9 months to give alcohol blood levels of about 2 mg/ml or by exposing the rats to ethanol vapor for

5 hours per day for 7 weeks to give blood alcohol levels of about 6 mg/ml, did not affect the MAO-A activity (Wiberg, Wahlström, and Oreland 1977). This result was recently confirmed by Romanova (1980).

A recent animal model for the use of alcohol has been the development of rat strains based on voluntary alcohol consumption (Eriksson 1969, 1980). However, no significant differences between the MAO-A or MAO-B activities in the brains of the alcohol-preferring rats compared with the alcohol-avoiding rats were found (Oreland et al. submitted for publication).

Is the Low Brain and Platelet MAO in Alcoholics a Primary or a Secondary Phenomenon?

Fundamental for the interpretation of the results with low MAO activities both in brain and in platelets being associated with alcohol abuse is the question of whether or not the low activity is a constitutional factor or is induced by the presence of alcohol in the body. This question recently has again been stressed by Belmaker et al. (1980). With respect to the platelet enzyme, all of the most immediate causes for a reduced activity can be ruled out (see above). But, on the other hand, an effect on the megakaryocyte formation in the bone marrow by chronic alcohol abuse for many years cannot be ruled out. The findings of low platelet MAO activities also in individuals with cycloid psychosis (Eckert et al. 1980) or suicidal behavior (Buchsbaum et al. 1977; Gottfries et al. in press) would tend to support the concept of a genetic regulation of platelet MAO rather than an influence of some inhibitory factor present secondary to certain disease states. Of those diseases, ethanol abuse and cycloid psychosis have been shown to have a genetic component in their etiopathogenesis (e.g., Bohman 1978; Goodwin 1979; Perris 1974). Furthermore, low platelet MAO activity in healthy volunteers as well as in depressed patients has been found to be correlated with a number of behavioral parameters, such as sensation seeking (Fowler, von Knorring, and Oreland in press; Murphy et al. 1977; Schooler et al. 1978) and monotony avoidance (Perris et al. 1980). Those studies are particularly important in the discussion of the origin of low platelet MAO in alcoholics, since sensation seeking and monotony avoidance are personality traits associated with alcohol abuse (Zuckerman 1979).

Additional support for the concept that the low platelet MAO activity in alcoholics is a constitutional characteristic is the finding of low MAO-A activity in some parts of the brain of alcoholics (see above). It has been shown in animal experiments that brain MAO activities are unchanged by a variety of environmental and nutritional factors and with chronic ethanol treatment (see above and Oreland et al. in press). Because of the parallelism in the findings of low platelet and brain MAO activity and also in suicidal behavior and cycloid psychosis (Eckert et al. 1980; Gottfries et al. 1975, in press; Wiberg 1978), it is tempting to postulate that brain and platelet MAO activities are regulated by some common, possibly genetic, mechanism. Naturally, a common genetic control of a platelet enzyme and a brain enzyme would not be easy to understand, but there are a number of other similarities between platelets and, for example, synaptosomes that are difficult to explain by chance, and a common embryonic origin has indeed been suggested (Campbell et al. 1980).

Why Do Alcoholics Have Low Platelet MAO Activity?

If it is assumed, as has been suggested in the foregoing discussion, that the low platelet MAO in alcoholics is a constitutional characteristic, then alcoholics should be included in the low platelet MAO, high risk paradigm originally proposed by the Murphy-Buchsbaum group (Buchsbaum et al. 1976). That is, low platelet MAO is a biochemical marker for certain personality traits, which in combination with certain other personality traits and a particular social setting will result in a certain psychiatric disorder. The multifactorial background of alcohol abuse has already been exemplified in one way by von Knorring and Oreland (1978), who found that augmenting the tendency of the visual evoked response together with low platelet MAO activity much better identified an alcoholic than did low platelet MAO activity alone. The recent finding of significant correlations between human platelet MAO in healthy volunteers and their concentrations of 5-HIAA and HVA in cerebrospinal fluid using univariated analyses (Oreland, Wiberg, Åsberg, Träskman, Sjöstrand, Thorén, Bertilsson, and Tybring in press) and concentrations of 5-HIAA, HVA, and MHPG in depressed patients (unipolar and bipolar) using multivariate analyses (Ågren and Oreland in preparation) seem to indicate that platelet MAO is a marker for central monoaminergic activity. Like the findings for platelet MAO activity (see above), there is indirect evidence of a genetic component for levels of 5-HIAA and HVA in CSF (Sedvall et al. in press; van Praag and de Haan in press). If the common denominator for platelet (and brain) MAO activity and central monoaminergic activity is the genetic control, then size or capacity of the monoaminergic system rather than actual monoamine transmitter substance turnover (as reflected by levels of metabolites in the cerebrospinal fluid) would be the relevant factor. A correlation between the size (number of neurones) of the dopaminergic system and the activity of tyrosine hydroxylase in mouse brain has previously been demonstrated by Reis et al. (1979). Variations in brain MAO activity within the range found in various patient categories (Eckert et al. 1980) and controls (Fowler, Oreland, Marcusson, and Winblad 1980; Fowler, Wiberg, Oreland, Marcusson, and Winblad 1980; Fowler, Wiberg, Oreland, and Winblad 1980) are not likely to affect the degradation of, e.g., serotonin to 5-HIAA, at least as indicated by animal experiments (see Green and Grahame-Smith 1978). Extra support for the notion that platelet MAO and, e.g., central serotonin turnover are related comes from the observation that differences in the rates of central serotonin turnover in different strains of mice are strongly correlated with different degrees of aggressive behavior (Valzelli and Bernasconi 1979). Furthermore, in humans, central serotonin turnover has also been implicated in the expression of certain personality traits (Brown et al. 1979; for a review, see Träskman-Bendz 1980). In conclusion, therefore, low platelet MAO activity may act as a marker for a low central monoaminergic "capacity," which in combination with other factors might dispose for alcohol abuse. If this is true, the implications for diagnosis as well as for treatment of this disease are easily anticipated.

Summary

The activity of human platelet and brain MAO is lower in alcoholics than in controls. These results and others indicate a common control of brain and platelet MAO, which would support a

recent hypothesis that there is a common ontogenetic origin of platelets and brain tissue. Platelet MAO activity is also correlated with CSF 5-HIAA and HVA, consistent with the hypothesis that low platelet MAO activity reflects a "weak" brain monoaminergic system. Thus, platelet and brain MAO could be regulated by the same control system also regulating the size of the monaminergic systems involved. The monoaminergic activity might then be of importance for constitutional factors predisposing for alcohol abuse.

References

Adolfsson, R.; Gottfries, C.G.; Oreland, L.; Wiberg, Å.: and Winblad, B. Increased activity of brain and platelet monoamine oxidase activity in dementia of Alzheimer type. *Life Sci*, 27:1029-1034, 1980.

Agarwal, D.P.; Goedde, H.W.; and Schrappe, O. Blood platelet monoamine oxidase activity in schizophrenia, affective disorders and alcoholism. In: Singer, T.P.; Von Korff, R.W.; and Murphy, D.L., eds. *Monoamine Oxidase: Structure, Function, and Altered Functions*. New York: Academic Press, 1979. pp. 397-402.

Athen, D.; Beckmann, H.; Ackenheil, M.; and Markianos, M. Biochemical investigations into the alcoholic delirium: Alterations of biogenic amines. *Arch Psychiatr Nervenkr*, 224:129-140, 1977.

Belmaker, R.H.; Bracha, H.S.; and Ebstein, R.P. Platelet monoamine oxidase in affective illness and alcoholism. *Schizophr Bull*, 6(2):320-323, 1980.

Bergman, H.; Borg, S.; Hindmarsh, T.; Ideström, C.-M.; and Mützell, S. Computed tomography of the brain and neuropsychological assessment of male alcoholics. In: Richter, D., ed. *Addiction and Brain Damage*. London: Croom Helm, 1980. pp. 201-214.

Bohman, M. Some genetic aspects of alcoholism and criminality. A population of adoptees. *Arch Gen Psychiatry*, 35:269-276, 1978.

Brown, G.L.; Goodwin, F.K.; and Ballenger, J.C. Aggression in humans correlates with cerebrospinal fluid amine metabolites. *Psychiatr Res*, 1:131-139, 1979.

Buchsbaum, M.S.; Coursey, R.D.; and Murphy, D.L. The biochemical high risk paradigm. Behavioural and familial correlates of low platelet monoamine oxidase activity. *Science*, 194:339-341, 1976.

Buchsbaum, M.S.; Haier, R.J.; and Murphy, D.L. Suicide attempts, platelet monoamine oxidase and the average evoked response. *Acta Psychiatr Scand*, 56:69-79, 1977.

Campbell, I.C.; Marangos, P.J.; and Murphy, D.L. Neurone specific enolase in platelets: Implications for the neuronal model. In: Radouco-Thomas, C., and Garcin, F., eds. *Abstracts of the 12th CINP Congress*, Göteborg, Sweden, 1980. Oxford: Pergamon Press, 1980. p. 99.

Carlsson, A.; Adolfsson, R.; Aquilonius, S.-M.; Gottfries, C.G.; Oreland, L.; Svennerholm, L.; and Winblad, B. Biogenic amines in human brain in normal aging, senile dementia and chronic alcoholism. In: Goldstein, M., et al., eds. *Ergot*

Compounds and Brain Function: Neuroendocrine and Neuropsychiatric Aspects. New York: Raven Press, 1980. pp. 295-304.

Donnelly, C.H., and Murphy, D.L. Substrate- and inhibitor-related characteristics of human platelet monoamine oxidase. *Biochem Pharmacol*, 26:853-858, 1977.

Eckert, B.; Gottfries, C.G.; von Knorring, L.; Oreland, L.; Wiberg, Å.; and Winblad, B. Brain and platelet monoamine oxidase in mental disorders. 1. Schizophrenics and cycloid psychotics. *Prog Neuropsychopharmacol*, 4:57-68, 1980.

Eriksson, K. Factors affecting voluntary alcohol consumption in the albino laboratory rat. *Ann Zool Fennici*, 6:227-265, 1969.

Eriksson, K. Inherited metabolism and behavior towards alcohol: Critical evaluation of human and animal research. In: Eriksson, K.; Sinclair, J.D.; and Kiianmaa, K., eds. *Animal Models in Alcohol Research*. London: Academic Press, 1980. pp. 3-20.

Fowler, C.J.; Ekstedt, B.; Egashira, T.; Kinemuchi, H.; and Oreland, L. The interaction between human platelet monoamine oxidase, its monoamine substrates and oxygen. *Biochem Pharamcol*, 28:3063-3068, 1979.

Fowler, C.J.; Oreland, L.; Marcusson, J.; and Winblad, B. Titration of human brain monoamine oxidase -A and -B by clorgyline and 1-deprenil. *Naunyn Schmiedebergs Arch Pharmacol*, 311:263-272, 1980.

Fowler, C.J.; Oreland, L.; Wiberg, Å.; Carlsson, A.; and Magnusson, T. The inhibition of rat brain monoamine oxidase-B by J-508 (N-methyl-N-propargyl-(1-indanyl)-ammonium hydrochloride), and its use for the titration of this enzyme form. *Med Biol*, 57:406-411, 1979.

Fowler, C.J.; von Knorring, L.; and Oreland, L. Platelet monoamine oxidase activity in sensation seekers. *Psychiatr Res*, in press.

Fowler, C.J., and Wiberg, Å. Molecular characteristics of human platelet monoamine oxidase. *Naunyn Schmiedebergs Arch Pharmacol*, 313:77-84, 1980.

Fowler, C.J.; Wiberg, Å.; Oreland, L.; Danielsson, Å.; Palm, U.; and Winblad, B. Monoamine oxidase activity and kinetic properties in platelet-rich plasma from controls, chronic alcoholics and patients with non-alcoholic liver disease. *Biochem Med*, in press.

Fowler, C.J.; Wiberg, Å.; Oreland, L.; Marcusson, J.; and Winblad, B. The effect of age on the activity and molecular properties of human brain monoamine oxidase. *J Neural Transm*, 49:1-20, 1980.

Fowler, C.J.; Wiberg, Å.; Oreland, L.; and Winblad, B. Titration of human brain type-B monoamine oxidase. *Neurochem Res*, 5(7), 1980.

Goodwin, D.W. Alcoholism and heredity. A review and hypothesis. *Arch Gen Psychiatry*, 36:57-61, 1979.

Gottfries, C.G.; Oreland, L.; Wiberg, Å.; and Winblad, B. Lowered monoamine oxidase activity in brains from alcoholic suicides. *J Neurochem*, 25:667-673, 1975.

Gottfries, C.G.; von Knorring, L.; and Oreland, L. Platelet monoamine oxidase activity in patients with suicidal behaviour and in subgroups of affective psychoses. *Prog Neuropsychopharmacol*, in press.

Green, A.R., and Grahame-Smith, D.G. Processes regulating the functional activity of brain. 5-hydroxytryptamine: Results of animal experimentation and their relevance to the understanding and treatment of depression. *Pharmakopsychiat*, 11:3-16, 1978.

Johnston, J.P. Some observations on a new inhibitor of monoamine oxidase in brain tissue. *Biochem Pharmacol*, 17:1285-1297, 1968.

Knoll, J., and Magyar, K. Some puzzling pharmacological effects of monoamine oxidase inhibitors. *Adv Biochem Psychopharmacol*, 5:393-408, 1972.

Major, L.F., and Murphy, D.L. Platelet and plasma monoamine oxidase activity in alcoholic individuals. *Br J Psychiatry*, 132:548-554, 1978.

MacPike, A.D., and Meier, H. Genotype dependence of monoamine oxidase in inbred strains of mice. *Experientia*, 32:979-980, 1976.

Murphy, D.L. Clinical, genetic, hormonal and drug influences on the activity of human platelet monoamine oxidase. In: Wolstenholme, G.E.W., and Knight, J., eds. *Monoamine Oxidase and Its Inhibition*, Ciba Foundation Symposium 39, Elsevier. Amsterdam: Exerpta Medica, North Holland, 1976. pp. 341-351.

Murphy, D.L.; Belmaker, R.H.; Buchsbaum, M.S.; Martin, N.F.; Ciaranello, R.; and Wyatt, R.J. Biogenic amine-related enzymes and personality variations in normals. *Psychol Med*, 7:149, 1977.

Murphy, D.L.; Wright, C.; Buchsbaum, M.S.; Nichols, A.; Costa, J.L.; and Wyatt, R.J. Platelet and plasma amine oxidase in 680 normals: Sex and age differences and stability over time. *Biochem Med*, 16:254-265, 1976.

Nies, A.; Robinson, D.S.; Lamborn, K.R.; and Lampert, R.P. Genetic control of platelet and plasma monoamine oxidase activity. *Arch Gen Psychiatry*, 28:834-838, 1973.

Oreland, L. The activity of human brain and thrombocyte monoamine oxidase (MAO) in relation to various psychiatric disorders. In: Singer, T.; von Korff, R.; and Murphy, D.L., eds. *Monoamine Oxidase: Structure, Function, and Altered Functions*. New York: Academic Press, 1979. pp. 379-387.

Oreland, L. Monoamine oxidase and affective illness. *Acta Psychiatr Scand*, 61(280):41-46, 1980.

Oreland, L.; Fowler, C.J.; Carlsson, A.; and Magnusson, T. The effect of hemitransection of rats upon the brain monoamine oxidase MAO-A and MAO-B activity. *Life Sci*, 26:139-146, 1980.

Oreland, L.; Gottfries, C.G.; Kiianmaa, K.; Wiberg, Å.; and Winblad, B. The activity of monoamine oxidase A and B in brains from chronic alcoholics. Submitted for publication.

Oreland, L.; Wiberg, Å.; Åsberg, M.; Träskman, L.; Sjöstrand, L.; Thorén, P.; Bertilsson, L.; and Tybring, G. Platelet MAO activity and monoamine metabolites in cerebrospinal fluid in depressed and suicidal patients and in healthy controls. *Psychiatr Res*, in press.

Oreland, L.; Wiberg, Å.; and Fowler, C.J. Monoamine oxidase activity in platelets as related to monoamine oxidase activity and monoaminergic function in the brain. In: *Recent Advances in Neuropsychopharmacology: Selected Papers from the 12th CINP Congress*. Oxford: Pergamon Press, in press.

Owen, F.; Acker, W.; Bourne, R.C.; Frith, C.D.; and Riley, G.J. The effect on human monoamine oxidase activity of subcutaneous injections of adrenaline. *Biochem Pharmacol*, 26:2065-2067, 1977.

Pandey, G.N.; Dorus, E.; Shaughnessy, R.; and Davis, J.M. Genetic control of platelet monoamine oxidase activity: Studies on normal families. *Life Sci*, 25:1173-1178, 1979.

Perris, C. A study of cycloid psychoses. *Acta Psychiatr Scand*, Suppl., 253:7-74, 1974.

Perris, C.; Jacobsson, L.; von Knorring, L.; Oreland, L.; Perris, H.; and Ross, S.B. Enzymes related to biogenic amines metabolism and personality characteristics in depressed patients. *Acta Psychiatr Scand*, 61:477-484, 1980.

Reis, D.J.; Baker, H.; Fink, J.S.; and Joh, T.H. A genetic control of the number of central dopamine neurons in relationship to brain organization, drug responses, and behavior. In: Usdin, E.; Kopin, I.J.; and Barchas, J., eds. *Catecholamines: Basic and Clinical Frontiers.* New York: Pergamon Press, 1979. pp. 23-33.

Romanova, L.A. Effect of acute and chronic administration of ethanol on monoamine oxidase activity in rat brain and liver tissues. *Vopr Med Khim,* 26:252-255, 1980.

Roth, J.A. Evidence for a single catalytic binding site on human brain type B monoamine oxidase. *J Neurochem,* 27:1107-1112, 1976.

Schooler, C.; Zahn, T.P.; Murphy, D.L.; and Buchsbaum, M.S. Psychological correlates of monoamine oxidase activity in normals. *J Nerv Ment Dis,* 166:177-186, 1978.

Sedvall, G.; Fryö, B.; Gullberg, B.; et al. Relationships in healthy volunteers between concentrations of monoamine metabolites in cerebrospinal fluid and family history of psychiatric morbidity. *Br J Psychiatry,* in press.

Student, A.K., and Edwards, D.J. Subcellular localization of type A and B monoamineoxidase in rat brain. *Biochem Pharmacol,* 26:2337-2342, 1977.

Sullivan, J.L.; Stanfield, C.N.; Maltbie, A.A.; Hammett, E.; and Cavenar, J.O. Stability of low blood platelet monoamine oxidase activity in human alcoholics. *Biol Psychiatry,* 13:391-397, 1978.

Träskman-Bendz, L. "Depression and Suicidal Behaviour—A Biochemical and Pharmacological Study." Unpublished M.D. thesis, Karolinska Institute, Stockholm, Sweden, 1980.

Valzelli, L., and Bernasconi, M. Aggressiveness by isolation and brain serotonin turnover changes in different strains of mice. *Neuropsychobiology,* 5:129-135, 1979.

van Praag, H.M., and de Haan, S. Central serotonin deficiency—A factor which increases depression vulnerability? *Acta Psychiatr Scand,* in press.

von Knorring, L., and Oreland, L. Visual averaged evoked responses and platelet monoamine oxidase activity as an aid to identify a risk group for alcoholic abuse. A preliminary study. *Prog Neuropsychopharmacol,* 2(3):385-392, 1978.

White, H.L., and Glassman, A.T. Multiple binding sites of human brain and liver monoamine oxidase: Substrate specificities, selective inhibitors, and attempts to separate enzyme forms. *J Neurochem,* 29:987-997, 1977.

Wiberg, Å. Umea University medical dissertations, New Series, No. 41, Umea, Sweden, 1978.

Wiberg, Å. Increase in platelet monoamine oxidase activity during controlled abstinence after alcohol abuse. *Med Biol,* 57:133-134, 1979.

Wiberg, Å.; Gottfries, C.G.; and Oreland, L. Low platelet monoamine oxidase activity in human alcoholics. *Med Biol,* 55:181-186, 1977.

Wiberg, Å.; Wahlström, G.; and Oreland, L. Brain monoamine oxidase activity after chronic ethanol treatment of rats. *Psychopharmacology,* 52(2):111-114, 1977.

Winter, H.; Herschel, M.; Propping, P.; Friedl, W.; and Vogel, F. A twin study on three enzymes (DBH, COMT, MAO) of catecholamine metabolism. Correlations with MMPI. *Psychopharmacology,* 57:63-69, 1978.

Wyatt, R.J.; Potkin, S.G.; Bridge, T.P.; Phelps, B.H.; and Wise, C.D. Monoamine oxidase in schizophrenia: An overview. *Schizophr Bull,* 6(2):199-207, 1980.

Yang, H.-Y.T., and Neff, N.H. β-phenethylamine: A specific substrate for type B monoamine oxidase of brain. *J Pharmacol Exp Ther,* 187:365-371, 1973.

Yu, P.H., and Boulton, A.A. Activation of platelet monoamine oxidase by plasma in the human. *Life Sci*, 25:31-36, 1979.

Zuckerman, M. *Sensation Seeking: Beyond the Optimal Level of Arousal.* Hillsdale, N.J.: Lawrence Erlbaum Associates, 1979.

Predictive Value of Platelet Monoamine Oxidase Activity in the Treatment of Depressed Alcoholics With Lithium

John L. Sullivan, Paula D. Sullivan, Jonathan Davidson, C. Edward Coffey, Steven Mahorney, Ronald J. Taska, and Jesse O. Cavenar

Introduction

Although it is widely appreciated that monoamine oxidase (MAO) serves an important oxidative function in amine metabolism, the relationship of changes in the structure and function of the enzyme, as well as changes in the enzyme's molecular environment, to human disease states remains a subject of considerable controversy. More specifically, the possible association between changes in MAO activity and psychopathology has proved to be one of the topical areas of investigative interest in psychobiology since Murphy and Wyatt (1972) reported a reduction in MAO activity in platelets from schizophrenic patients. Many of the studies on MAO activity in psychopathological states reported in the literature since then have been with schizophrenic subjects. In one of the most recent comprehensive reviews of MAO activity in schizophrenia, Wyatt and associates (1979) indicated that 19 of 26 studies reported in the literature showed a statistically significant decrease in MAO activity in the platelets and white cells of chronic schizophrenics. The probability that this figure can be obtained by chance alone

---

Note: Tables appear at end of paper.

was calculated as less than 1 in 10^{19} (binomial test). A number of other studies have also shown an association between low platelet MAO activity and bipolar affective illness or alcoholism (Belmaker et al. 1980).

However, the biological significance of reduced MAO activity in the blood cellular elements of some mentally ill individuals, including chronic schizophrenics, is still uncertain. Although platelet MAO activity is a relatively stable characteristic and heritability is a major determinant of MAO activity, a number of metabolic factors appear to influence the enzyme, including the platelet form (Sullivan et al. 1980). These metabolic factors include ontogenic development, aging, hormonal influence, diet, iron deficiency, riboflavin deficiency, medication (including a variety of psychotropic agents), anxiety, stress, activity of other oxidative enzymes, endogenous inhibitors and activators, the lipid environment of the enzyme, immunologic status, and coexistent medical illnesses, including infectious diseases.

In efforts to elucidate the psychobiological significance of reduced MAO activity in psychiatric illness, other investigators have also studied the association between low levels of the enzyme and specific behaviors. For example, Buchsbaum and colleagues (1976) showed an association between low platelet MAO activity and psychiatric illness in a study of college student volunteers, in which individuals with low platelet MAO activity had a higher incidence of psychiatric illness, as well as a higher incidence of suicide and suicide attempts in their families, compared with subjects with high platelet MAO activity. Schildkraut et al. (1976) and Meltzer (1977) suggested an association between low platelet MAO activity and auditory hallucinations. Takahashi et al. (1976) reported that platelet MAO activity was low in alcoholics with delirium tremens, and Gottfries and his collaborators (1974, 1975) demonstrated a relationship between low MAO activity in the brain and suicidal behavior in alcoholics. Wyatt and associates (1978) indicated an association between low platelet enzyme activity and paranoia. Sullivan, Maltbie, Cavenar et al. (1978) and Sullivan, Stanfield, Schanberg, and Cavenar (1978) subsequently suggested an association between impaired oxidative metabolism reflected in low MAO activity and psychopathology with a prominent affective component.

Sullivan, Cavenar, Maltbie, and Stanfield (1978) also studied the relationship between platelet MAO activity and lithium response in manic depressives. The results of the study indicated that acutely ill bipolar subjects with low platelet MAO activity demonstrated a poor therapeutic response to lithium compared with subjects with normal to high enzyme activity. On the basis of these findings, it appeared that measurement of platelet MAO activity could possibly prove useful as a clinical laboratory aid for lithium therapy. Therefore, the following study was designed to examine the relationship between platelet MAO activity and lithium response in depressed alcoholics in order to provide further observations in this area.

Methods

Subject Selection

The 71 alcoholics who began the study (table 1) were selected from a medical outpatient clinic population. These individuals were males between the ages of 30 and 40 years (33.1±1.6 mean±SEM) who met the Research Diagnostic Criteria (RDC) (14) for alcoholism and also demonstrated a history of excessive drinking for at least 5 years. These individuals were primary alcoholics in that "the first major life problem related to alcohol occurred in an individual who had no existing psychiatric disorder" (Schuckit 1979). Each subject was followed in the medical clinic primarily for secondary depression, in the sense that the diagnosis of alcoholism antedated the diagnosis of depression. The affective disturbance was also present following abstinence from alcohol for at least 4 weeks. The diagnosis of primary alcoholism was confirmed by interviews with family members, which included at least one biological parent or full sibling, as well as review of previous psychiatric and medical records. None of the alcoholic subjects suffered from serious chronic or coexistent medical illness at the time of the study, including hematologic, neurologic, or dermatologic abnormalities compatible with iron deficiency anemia or riboflavin deficiency. In addition, there was no evidence on interview or review of medical and psychiatric documents of psychiatric diagnosis other than primary alcoholism and secondary depression. The degree of the depression was assessed with the Zung (1965) Self-Rating Depression Scale and the Hamilton (1960) Depression Scale. Two raters were used for the Hamilton scale with an interrater correlation of 0.86. Both raters were blind as to which subjects were receiving lithium and which subjects were receiving placebo. The Hamilton scale ratings for each subject represent the mean value for the two raters.

Candidates for the study were screened after they had been followed in the clinic for at least 4 weeks on a regular basis, in order to minimize noncompliance with the study. If they agreed to participate in the study and met the necessary criteria, all medication they were taking was discontinued. Also, they were scheduled for clinic visits at least every other week, free of physician's fees. In addition to the patient, at least one family member familiar with the patient's daily activities was contacted at the time of each followup to verify abstinence from alcohol during the study. Baseline behavioral ratings for depression and blood sampling were obtained 4 weeks after the patient agreed to participate in the study and discontinued any medication. All patients in the study received supportive psychotherapy for the duration of the study in addition to some form of pharmacotherapy.

The 96 male control subjects in the study were selected from university students, university employees, and hospital employees between the ages of 30 and 40 years (36.1±1.4, mean±SEM). Each control subject denied psychiatric illness or chronic medical illness and had not taken medication for at least 4 weeks prior to blood sampling.

Platelet MAO Assay

Venous blood samples (18 ml) were collected with a 19-gage needle into a plastic syringe, and platelet pellets were prepared according to the method described by Corash (1980). The pellets were homogenized to obtain the enzyme preparations for assay. Enzyme activity was assayed with liquid scintillation spectrometry with ^{14}C-tryptamine as substrate, using a modification of the method described by Wurtman and Axelrod (1963). Specific details of the assay procedure are published elsewhere (Sullivan et al. 1977).

Pharmacotherapy

To assess the potential usefulness of platelet MAO activity as an aid in lithium therapy for depressed alcoholics, the alcoholic subjects in the study were divided into two groups on the basis of their baseline platelet MAO activity (table 2). The low platelet

MAO activity group consisted of individuals with baseline enzyme activity of 50 percent or less of the mean enzyme activity for the control population. The other group of alcoholics consisted of subjects with normal to high baseline platelet MAO activity, in that individual enzyme activities were greater than 50 percent of the mean enzyme activity for the control population.

Each of these two groups was further randomly divided into two subgroups of approximately even number so that each subgroup could be treated with either lithium capsules or matching inert placebo capsules on a double-blind basis. For the subgroups that received lithium, medication was adjusted to achieve a serum lithium level range of 0.8 mEq/l to 1.2 mEq/l with conventional clinical laboratory analysis. Patients on placebo received a similar number of capsules. In addition, blood samples were drawn on patients whether they were receiving lithium or placebo, and placebo medication was adjusted to help maintain the double-blind status of the study. The investigator dispensing the medications did not participate in the psychiatric evaluations of the patients.

Pharmacotherapy was initiated following baseline depression ratings and blood sampling for platelet MAO activities. The behavioral ratings were repeated every 4 weeks for the 12-week duration of the study of each patient. Patients who did not complete the 12-week course of the study were regarded as treatment failures and dropped from the study.

Criteria for admission to the study, as well as criteria for improvement and worsening in clinical condition, are summarized in table 3. Improvement included continued abstinence from drinking alcohol in addition to improvement in affective status as reflected by a minimum decrease of greater than 10 points in the Zung SDS index and/or mean Hamilton scale rating. A worse clinical status included relapse to alcohol consumption and/or worsening of depression as reflected by a minimum increase of greater than 10 points in the Zung SDS index and/or mean Hamilton scale rating.

Results

The results of lithium versus placebo treatment in depressed alcoholics with low platelet MAO activity (Zung SDS and Hamilton scale) are outlined in table 4. Essentially, neither placebo nor lithium resulted in significant improvement in these subjects. Tables 5 and 6 outline the results of lithium versus placebo treatment in depressed alcoholics with normal to high platelet MAO activity. When the Zung SDS is used as a criterion for change, no significant improvement is noted in either subgroup at 4 weeks. However, at 8 weeks and 12 weeks following initiation of pharmacotherapy, the lithium-treated subgroup showed significant improvement in its depression ratings ($p < .01$, chi-square) compared with the placebo-treated subgroup. Similar results were obtained when the Hamilton scale was used to assess depression ($p < .05$, chi-square). There were essentially no differences between subgroups among the nine subjects who dropped out of the study.

Discussion

The results of this study suggest a relationship between platelet MAO activity and lithium treatment outcome in depressed alcoholics. Essentially, abstinent depressed alcoholics with normal to high platelet MAO activity showed significant and sustained improvement in their levels of depression, together with continued abstinence from alcohol, from 8 to 12 weeks following initiation of lithium therapy, compared with those receiving placebo pharmacotherapy. This relationship was not apparent in abstinent depressed alcoholics with low platelet MAO activity. The results of this study confirm and extend previous work that indicates that low platelet MAO activity in psychiatrically ill individuals with a prominent affective component to their illness is associated with a poor response to lithium therapy. Although there is no precise explanation for this phenomenon, Carroll (1980) has suggested that genetically determined personality factors and predisposition to psychopathology linked to low platelet MAO activity could adversely influence lithium treatment response.

Although it appears that lithium treatment does not directly affect MAO activity, some studies show that lithium administration can produce increased amine uptake in brain and in human platelets together with decreased amine release (Murphy and Weiss 1972). Some of the consequences of these metabolic changes, such as increased formation of deaminated metabolites, may be antagonized by defective oxidative metabolism associated with low MAO activity at critical tissue sites. It also appears that such a metabolic defect need not be initiated centrally in order to alter central nervous system function. As Wyatt and associates (1979) have previously indicated, phenylketonuria is a disease with prominent alterations in brain function where the primary metabolic abnormality is in peripheral tissues. Perhaps low MAO activity in some peripheral tissues facilitates the effects of potentially toxic metabolites, such as octopamine, on central neural transmission and lithium effect (Sullivan, Stanfield, Schanberg, and Cavenar 1978). However, it is clear that more experimental data are needed to clarify these complex issues of intermediary metabolism and potential pharmacologic influence. A cautionary note is that the usefulness of pharmacologic models in this area is complicated by previous reports that indicate that some depressed patients respond to treatment with lithium in combination with an MAO inhibitor but not to other antidepressant regimens (Schildkraut 1973). Furthermore, it is worth noting that the mean platelet MAO activity of the alcoholics in this study was essentially the same as the mean enzyme activity for the age-matched and sex-matched controls. One of the implications of this observation is that issues other than a mean difference in enzyme activity between chronic alcoholics and controls require attention in clinical investigations.

Unfortunately, the relatively short duration of our study design leaves a number of questions unanswered. For example, the relationship between platelet MAO activity and lithium prophylaxis against excessive drinking behavior in chronic alcoholics is still not clear. A critical issue within this context, which has been studied by Schuckit et al. (1979), is the relationship between alcoholism and affective illness. For example, the coexistence of depression and alcoholism in our study population could reflect the coexistence of two independent disorders. However, the expected concomitance of primary alcoholism with primary affective disorders (unipolar and bipolar) is in the range of 0.24 to 0.44 percent. Therefore, it seems unlikely that the results of this particular study can be explained solely on the basis of coexistent affective disorders and alcoholism, which are etiologically and pathogenetically dissimilar.

Interestingly, a 1-year prospective double-blind, placebo-controlled trial by Reynolds and associates (1979) indicated that nondepressive alcoholics showed no significant change on lithium as compared to placebo, whereas those patients rated as depressive showed a very significant decrease in drinking and alcoholic morbidity. There was also a greater improvement in the level of depression in the patients on lithium compared with those on placebo, suggesting an interrelationship between the effects of lithium on affective status and drinking behavior in depressed alcoholics. However, other investigators (Kline and Cooper 1979) have reported that lithium can have a statistically significant beneficial effect on the drinking behavior of chronic alcoholics in which there was a significant improvement in the depression ratings of both the placebo-treated and lithium-treated groups. These data support the likelihood that the effects of lithium on the behavior of chronic alcoholics are not uniform but rather subject to modifying factors. Perhaps some useful information on such potential modifying factors can be obtained by more extensive observations on the relationship between platelet MAO activity and lithium therapy in chronic alcoholics, particularly alcoholics with a prominent affective component to their illness, which is reflected in such behavioral indexes as depression and suicide attempts.

Table 1. Platelet Monoamine Oxidase (MAO) Activity in Alcoholic Experimental Subjects and Nonpsychiatric Controls

	Controls	Alcoholics
N	96	71
Age (mean ± SEM)	36.1 ± 1.4	33.9 ± 1.6
Sex	Male	Male
Platelet MAO activity (mean ± SEM)	11.1 ± 0.7 (nmol/10^9 platelets/hr)	10.5 ± 0.6 (nmol/10^9 platelets/hr)

Table 2. Platelet MAO Activity (Mean ± SEM) in Four Subgroups of Alcoholics Treated With Placebo or Lithium

	Low MAO Activity (nmol/10^9 platelets/hr)	Normal to High MAO Activity (nmol/10^9 platelets/hr)
Placebo-treated subgroup	4.2 ± 0.3 (*N* = 6)	12.5 ± 0.8 (*N* = 26)
Lithium-treated subgroup	4.4 ± 0.3 (*N* = 5)	12.2 ± 0.7 (*N* = 25)

Table 3. Criteria for Depression in Alcoholics and Change in Depression During Treatment (Zung SDS Index and Hamilton Scale Were Used to Assess Depression)

	Zung SDS Index	Hamilton Scale (17 Factor)
Admission to study (moderate depression)	60–69	25–33
Improved (normal range to mild depression)	< 59	< 14
Worse (severe depression)	> 70	> 34

Table 4. Results of Lithium Versus Placebo Treatment in Depressed Alcoholics With Low Platelet MAO Activity (Zung SDS Index and Hamilton Scale)

	Number of Subjects		
	Unchanged	Worse	Improved
Placebo			
4 weeks	5	1	0
8 weeks	4	1	1
12 weeks	5	0	1
Lithium			
4 weeks	5	0	0
8 weeks	4	0	1
12 weeks	3	1	1

Table 5. Results of Lithium Versus Placebo Treatment in Depressed Alcoholics With Normal to High Platelet MAO Activity (Zung SDS Index)

	Number of Subjects		
	Unchanged	Worse	Improved
Placebo			
4 weeks	22	2	2
8 weeks	20	0	6
12 weeks	18	2	6
Lithium			
4 weeks	21	2	2
8 weeks	7	2	16[a]
12 weeks	8	1	16[a]

[a] $p < .01$, chi-square.

Table 6. Results of Lithium Versus Placebo Treatment in Depressed Alcoholics With Normal to High Platelet MAO Activity (Hamilton Scale)

	Number of Subjects		
	Unchanged	Worse	Improved
Placebo			
4 weeks	20	1	5
8 weeks	18	2	6
12 weeks	18	2	6
Lithium			
4 weeks	21	2	2
8 weeks	9	2	14[a]
12 weeks	9	2	14[a]

[a] $p < .05$, chi-square.

References

Belmaker, R.H.; Bracha, H.S.; and Ebstein, R.P. Platelet monoamine oxidase in affective illness and alcoholism. *Schizophr Bull*, 6:320-323, 1980.

Buchsbaum, M.S.; Coursey, R.D.; and Murphy, D.L. The biochemical high-risk paradigm: Behavioral and familial correlates of low platelet monoamine oxidase activity. *Science*, 194:339-341, 1976.

Carroll, B.J. Prediction of treatment outcome with lithium. *Arch Gen Psychiatry*, 36:870-878, 1980.

Corash, L. Platelet heterogeneity: Relevance to the use of platelets to study psychiatric disorders. *Schizophr Bull*, 6:254-258, 1980.

Gottfries, C.G.; Oreland, L.; Wiberg, Å.; and Winblad, B. Brain-levels of monoamine oxidase in depression. *Lancet*, 2:360-361, 1974.

Gottfries, C.G.; Oreland, L.; Wiberg, Å; and Winblad, B. Lowered monoamine oxidase activity in brains from alcoholic suicides. *J Neurochem*, 25:667-673, 1975.

Hamilton, M. A rating scale for depression. *J Neurol Neurosurg Psychiatry*, 23:56-62, 1960.

Kline, N.S., and Cooper, T.B. Lithium therapy in alcoholism. In: Goodwin, D.W., and Erickson, C.K., eds. *Alcoholism and Affective Disorders*. Jamaica, New York: SP Medical and Scientific Books, 1979. pp. 21-29.

Meltzer, H.Y.; Pscheidt, G.R.; Goode, D.J.; et al. "Platelet Monoamine Oxidase and Plasma Amine Oxidase in Schizophrenia." Paper presented at the American Psychiatric Association Annual Meeting, Toronto, Canada, 1977.

Murphy, D.L., and Weiss, R. Reduced monoamine oxidase activity in blood platelets from bipolar depressed patients. *Am J Psychiatry*, 128:35-41, 1972.

Murphy, D.L., and Wyatt, R.J. Reduced monoamine oxidase activity in blood platelets from schizophrenic patients. *Nature*, 238:225-226, 1972.

Reynolds, C.M.; Merry, J.; and Coppen, A. Prophylactic treatment of alcoholism by lithium carbonate: An initial report. In: Goodwin, D.W., and Erickson, C.K., eds. *Alcoholism and Affective Disorders*. Jamaica, New York: SP Medical and Scientific Books, 1979. pp. 31-37.

Schildkraut, J.J. Pharmacology—The effects of lithium on biogenic amines. In: Gershon, S., and Shopsin, B., eds. *Lithium: Its Role in Psychiatric Research and Treatment*. New York: Plenum Press, 1973. pp. 51-73.

Schildkraut, J.J.; Herzog, J.M.; Orsulak, P.J.; et al. Reduced platelet monoamine oxidase activity in a subgroup of schizophrenic patients. *Am J Psychiatry*, 133:438-439, 1976.

Schuckit, M.A. Alcoholism and affective disorder: Diagnostic confusion. In: Goodwin, D.W., and Erickson, C.K., eds. *Alcoholism and Affective Disorders*. Jamaica, New York: SP Medical and Scientific Books, 1979. pp. 9-19.

Spitzer, R.L.; Endicott, J.; and Robins, E. *Research Diagnostic Criteria (RDC) for a Selected Group of Functional Disorders*. 3d ed. New York: New York Psychiatric Institute, Biometrics Research, 1977.

Sullivan, J.L.; Cavenar, J.O.; Maltbie, A.A.; and Stanfield, C.N. Platelet monoamine oxidase activity predicts response to lithium in manic-depressive illness. *Lancet*, 2:1325-1327, 1978.

Sullivan, J.L.; Coffey, C.E.; Sullivan, P.D.; et al. Metabolic factors affecting monoamine oxidase activity. *Schizophr Bull*, 6:308-313, 1980.

Sullivan, J.L.; Dackis, C.; and Stanfield, C.N. In vivo inhibition of platelet MAO activity by tricyclic antidepressants. *Am J Psychiatry*, 134:188-190, 1977.

Sullivan, J.L.; Maltbie, A.A.; Cavenar, J.O.; et al. Monoamine oxidase in schizophrenia. *N Engl J Med*, 298:1151, 1978.

Sullivan, J.L.; Stanfield, C.N. Schanberg, S.; and Cavenar, J.O. Serum dopamine-B-hydroxylase and platelet monoamine oxidase in chronic alcoholism. *Arch Gen Psychiatry*, 35:1209-1212, 1978.

Takahashi, S.; Tani, N.; and Yamane, H. Monoamine oxidase activity in blood platelets in alcoholism. *Folia Psychiatr Neurol Jpn*, 30:455-462, 1976.

Wurtman, R.J., and Axelrod, J. Sex steroids, cardiac ^{3}H-norepinephrine and tissue monoamine oxidase levels in the rat. *Biochem Pharmacol*, 12:1417-1419, 1963.

Wyatt, R.J.; Potkin, S.G.; and Murphy, D.L. Platelet monoamine oxidase activity in schizophrenia: A review of the data. *Am J Psychiatry*, 136:377-385, 1979.

Wyatt, R.J.; Potkin, S.G.; Walls, P.; et al. Clinical correlates of low platelet monoamine oxidase in schizophrenic patients. In: Akiskal, H., and Webb, W., eds. *Exploration of Biological Predictors*. New York: Spectrum Publications, 1978.

Zung, W.W.K. A self-rating depression scale. *Arch Gen Psychiatry*, 12:63-70, 1965.

Effect of Maternal Alcohol Ingestion on Developing Rat Brain MAO*

Samuel Eiduson and Regina Groshong

Introduction

A number of studies have shown that the concentration of biogenic amines such as serotonin, dopamine, and norepinephrine in the central nervous system are affected by ethanol or its metabolite (Gursey and Olson 1960; Renis et al. 1974; Rosenfeld 1960; Tabakoff 1977); however, the mechanism of these actions has yet to be determined and reasonably delineated. That MAO may be one of the possible sites of action of ethanol or acetaldehyde has been suggested by several researchers (Kurosawa 1974; Masamoto et al. 1974; Maynard and Schenker 1962; Towne 1964; Walsh et al. 1970), but the data in the literature are both inconclusive and controversial (Kumar and Rawat 1977). Alcohol has been shown to alter rat brain microsomal sulfhydryl groups (Dinovo et al. 1976) and to affect the red cell membrane by an appreciable extent (Seeman and Roth 1972). More recently, Cook and coworkers (1980) showed that dopamine binding sites in membranes from the corpus striatum of rats were altered by a diet containing ethanol. Furthermore, Reitz (1980) demonstrated that chronic ethanol altered the membrane lipids in brain, spinal cord, and other tissues, and Littleton and coworkers (1980) found an alteration of phospholipid composition in certain strains of mice. Reyes (1980) showed

---

Note: Figures and tables appear at end of paper.

*This work was supported in part by NIMH grant 19734-10 and NIAAA grant AA03513.

that alcohol altered the levels of a membrane-bound enzyme, gamma-glutamyl transpeptidase, and Chweh and Leslie (1980) showed effects on lysosomal membrane-bound arylsulfatase B.

Although there is still no appropriate animal model for the human fetal alcohol syndrome (FAS), a number of different animals have judiciously been employed in this regard. Because our laboratory has been interested in the normal developmental characteristics of the brain (Eiduson and Buckman 1979) for several years, we decided to use this interest to study the developing rat brain as a model for the fetal alcohol syndrome. Our interest centered on the development of brain monoamine oxidase. Because some references in the literature suggested that mitochondria of brains of animals administered alcohol were distorted (Karwaka et al. 1980; Kurosawa 1974; Masamoto et al. 1974), and because MAO is a component of the outer membrane of the mitochondria, we undertook to study the effect of maternal alcohol ingestion on developing rat brain MAO. (Several studies have shown an effect of in utero exposure to alcohol that altered a variety of neurotransmitters (Kumar and Rawat 1977; Rawat 1977; Thadani et al. 1977; Yanai et al. 1975) and enzymes (Branchey and Friedhoff 1973; Rawat 1977; Streissguth 1978; Thadani et al. 1977).

Method

Because MAO exists in at least two forms, A and B, having different substrate and inhibitor affinities, we looked at the development of these two forms in the brain. We used as substrates for the brain enzyme one that is preferentially metabolized by the A-form (5-hydroxytryptamine, 5HT), one that is metabolized by the B-form (phenylethylamine, PEA), and one that is metabolized by both forms (tryptamine). Additionally, we used two different inhibitors: clorgyline, which preferentially inhibits the A-form of the enzyme (at 10^{-7} M), and deprenyl, which preferentially inhibits the B-form (at 10^{-7} M). The enzyme assay employed was as described previously (Shih and Eiduson 1971, 1973).

To obtain alcoholic dams, female rats (several weeks prior to mating) were placed on regular rat chow (Purina), and 10 percent ethyl alcohol was added to the drinking water. Following mating, the female rats received a liquid diet (Diet 711-PR Bio-Xerv Inc., casein supplemented) (Taylor et al. in press). The pair-fed diets were the same except that an isocaloric amount of maltose-dextrin replaced the ethanol.

On day 8 of gestation, the dams were divided into three weight-matched groups for subsequent feeding of the three different diets: (a) liquid diet containing 5.0 percent w/v ethanol, ad libitum; (b) isocaloric liquid diet without ethanol, pair-fed to the amount consumed by the ethanol dams to control for nutritional deficits; or (c) normal lab chow and water, ad libitum.

Results

Figures 1, 2, and 3 show the results obtained when we inhibited normal developing brain MAO derived from animals of different ages, using the substrates and inhibitors mentioned above.

As figure 1 shows, with 5HT as substrate, clorgyline completely inhibited the A-substrate across all ages. That is, all A-sites of the enzyme were completely inhibited from newborn to adult. However, when the B-form inhibitor, deprenyl, was employed with 5HT as substrate, very little inhibition occurred. This result suggests that either no B-enzyme sites were present or 5HT does not readily serve as a substrate for the B-site.

As shown in figure 2, with tryptamine as substrate, it was observed that clorgyline inhibited the newborn brain MAO almost completely, with the inhibition due to the A-inhibitor declining with the age of the brain. Conversely, when the B-inhibitor was studied, it could be seen that deprenyl inhibited the metabolism of tryptamine by B-sites only minimally in the newborn brain, but then the ability of the B-inhibitor to affect the metabolism of the substrate was increased with increasing age of the brain. These clorgyline data suggested, therefore, that in the newborn brain, as indicated above, primarily A-sites prevailed but that B-sites became available as the brain matured and that, therefore, although the A-sites were blocked by clorgyline, the substrate could be metabolized by the developing B-sites and the inhibition relieved.

Conversely, deprenyl resulted in minimal inhibition of the metabolism of the substrate by the newborn, but this inhibition increased with the increasing age of the brain. Thus, these data suggested that B-sites of the enzyme increased with time and were therefore blocked by the inhibitor. This result was seen more dramatically when a B-substrate was used.

Figure 3 depicts the results obtained with PEA as substrate and when clorgyline was the inhibitor. Again, the MAO of the newborn brain was almost completely inhibited, but this inhibition by clorgyline dropped rapidly with developing brain. On the other hand, deprenyl inhibited the metabolism of the B-substrates only about 15 to 20 percent, but the inhibition of the metabolism of this substrate increased rapidly with increasing age. In fact, the two curves crossed at about 16 days of age. The data with clorgyline suggested that PEA could serve as an A-substrate and was completely inhibited in the newborn where the A-sites were predominant. Subsequently, this inhibitor activity decreased sharply with time as, apparently, the B-sites became available for metabolism of the PEA. In this event, as expected, deprenyl inhibited the metabolism of PEA more and more completely with increasing age.

The above results were some of the observations we have made on MAO activity in normal developing brain and served as one of the controls for assessing the effect of maternal alcohol ingestion on this development during the entire gestational period. The effect of maternal ingestion of alcohol on the developing rat brain MAO was then investigated using the same parameters as previously described. An additional control group, pair-fed with the alcohol group, was included for study. These results are shown in figures 4, 5, and 6.

In figure 4, the triangles represent the data obtained with the alcohol group, whereas the squares are data for the pair-fed controls. As can be seen, when 5HT was used as substrate, little difference was seen among all three groups.

Again, for figure 5 the triangles represent the alcohol group and squares represent the pair-fed controls. Although there were apparent differences between the alcohol and normal control groups, these differences disappeared when the nutritional effects were eliminated by comparing the alcohol and pair-fed controls.

These same observations were obtained when the B-substrate (PEA) was used, as seen in figure 6. Although differences between the normal control and the alcohol groups again existed, no such differences were seen when the alcohol group was compared with pair-fed controls.

Instead of plotting inhibition data as above, we plotted the specific activity of MAO over age as found in the experimental alcohol group and the two control groups, and results similar to those seen previously were obtained. Because the results were the same, the curves are not depicted.

In all of the experiments mentioned thus far, the concentration of substrates used was 200 μM. Since it was possible that this concentration of substrate could be inhibitory to the enzyme, particularly with PEA, we measured the activity of the enzyme over varying concentrations of substrate (10 μM to 200 μM). Figure 7 describes the data we obtained with our 40-day-old normal control group. Thus, we have plotted the specific activity (nmoles per 30 minutes per mg protein) of MAO, with and without the inhibitors, using PEA as substrate. These data showed that with a B-substrate, clorgyline had little effect on the MAO derived from 40-day-old brains over the concentration of substrate used. As expected, deprenyl had a greater effect over the range used. The effects of the inhibitors on the specific activities were additive, as seen in table 1.

These data clearly demonstrated that the activity remaining after each of the suicide inhibitors could be summated to yield the data seen with the control group. It is quite apparent that these inhibitors, at the concentrations of substrate and inhibitors used, act independently of each other.

Since it appeared that the activities of MAO following these suicide inhibitors could be additive and compared, we undertook to inhibit the enzyme preparation first with one inhibitor, then with the other. Using the 40-day-old normal control group mentioned above, we obtained the normative data shown in figure 8.

In this experiment, we incubated the enzyme for 30 minutes with either clorgyline or deprenyl, measured the inhibition, and then dialyzed away the excess inhibitor not bound to the enzyme. We then incubated this blocked enzyme once more with the same inhibitor over a varying concentration range of substrate (PEA). As shown in figure 8, the curves obtained either with single inhibition of the enzyme, or double inhibition, are clearly superimposable.

The family of inhibition curves seen (with PEA as substrate over a range of concentrations, figure 9) describes quite clearly the developmental characteristics of the A- and B-forms of the enzymes during development. These data also demonstrate, as suggested earlier, that at the early ages and limiting concentrations of PEA, the A-sites predominate and that PEA could readily serve as substrate and was inhibited by the A-inhibitor, clorgyline. As the concentration of substrate was increased, deprenyl had a greater effect and clorgyline had a lesser effect. We have not, however, completed the study using this paradigm with the alcohol and pair-fed controls.

To determine whether maternal alcohol ingestion affected neuronal MAO to a greater or lesser extent than non-neuronal enzyme, determinations were made on whole brain MAO as well as synaptosomal MAO. The results are shown in table 2. Although there were clear differences between the synaptosomal and whole brain preparation MAO levels, there were no differences observed between the normal control and alcohol groups.

Conclusions

The observed data presented on developing rat brains derived from "alcoholic" mothers strongly suggest that monoamine oxidase activity is not appreciably altered by the ingestion of alcohol by the dams. It should be noted that we were probably measuring the in utero effect, since the "alcoholic" and "pair-fed" pups were cross-fostered to normal dams at day 1 of birth. Although we may have missed some more possibly subtle effects (such as in specific brain regions), the lack of difference between normal whole brain MAO and enriched synaptosomal MAO in the control group when compared with that in the alcoholic and pair-fed groups suggested that, insofar as monoamine oxidase is concerned, the rat brain model for the fetal alcohol syndrome is inappropriate.

Figure 1. **Inhibition of Developing Rat Brain by Clorgyline and Deprenyl With 5HT as Substrate**

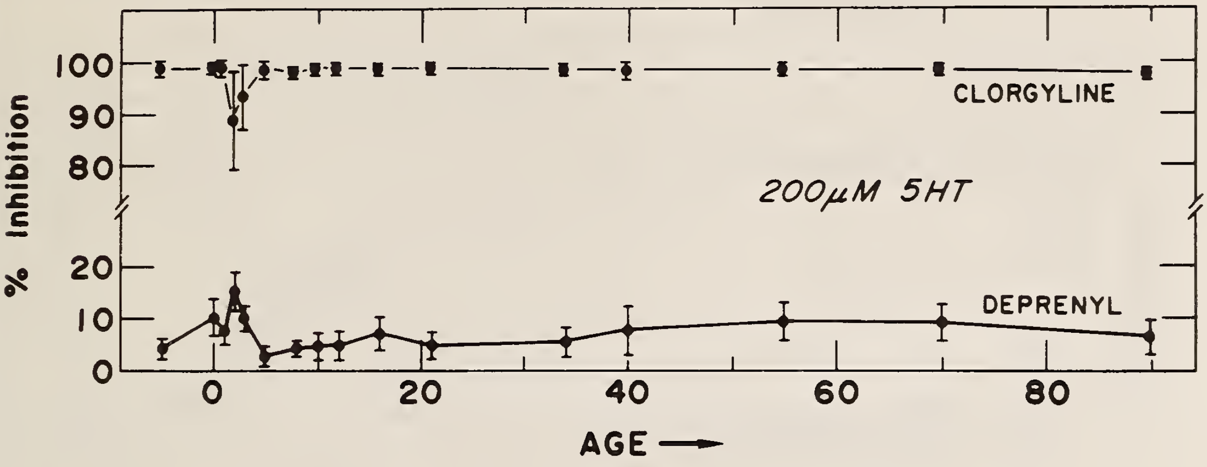


Figure 2. **Same as Figure 1 Except That Tryptamine Was the Substrate for the Enzyme**

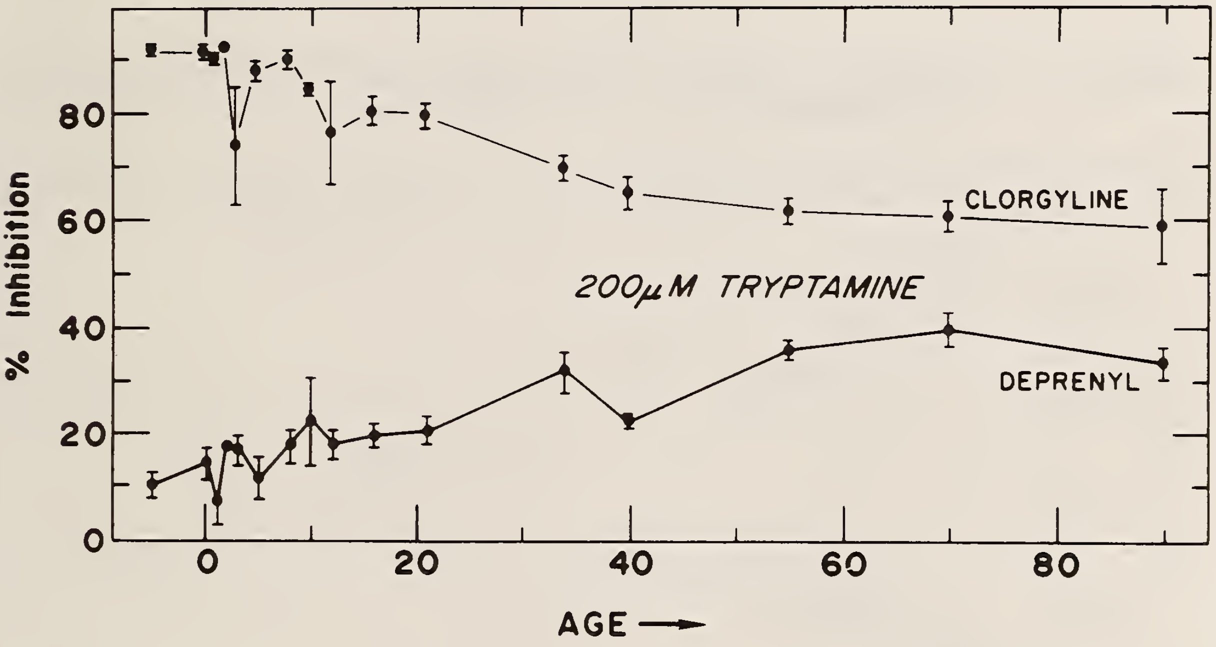


Figure 3. **Same as Figure 1 Except That PEA Was the Substrate for the Enzyme**

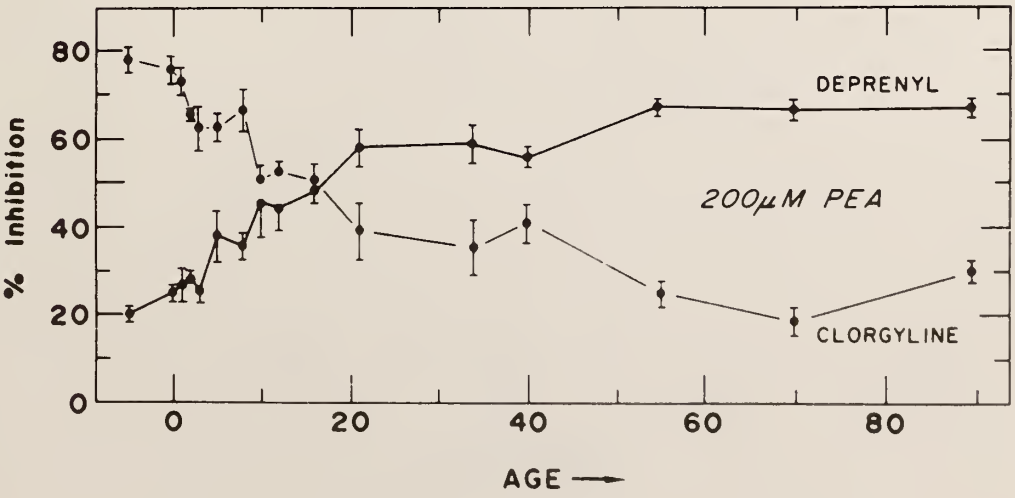


Figure 4. **Effect of Maternal Alcohol Ingestion on Developing Rat Brain MAO With 5HT as Substrate**

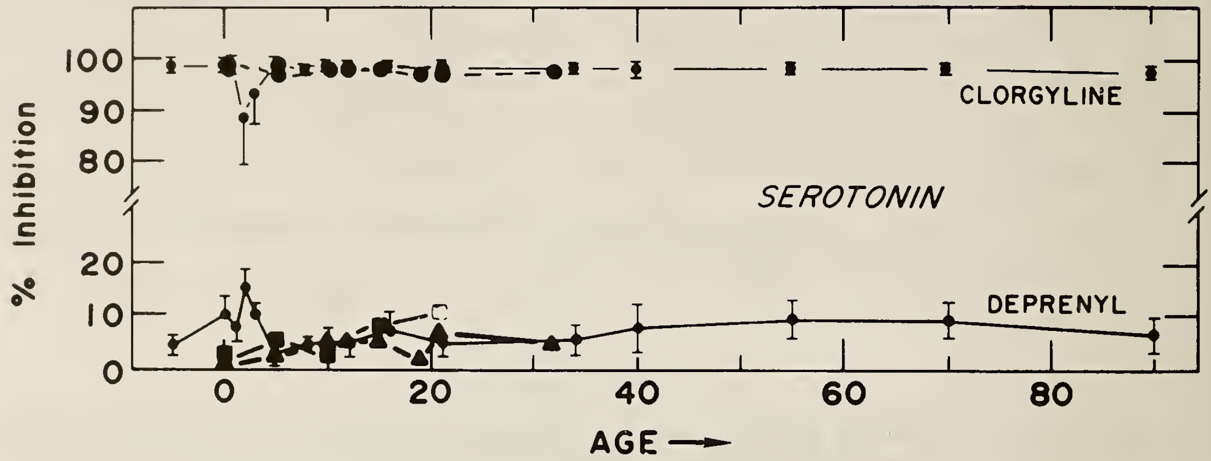


Figure 5. **Same as Figure 4 Except That Tryptamine Was the Substrate for the Enzyme**

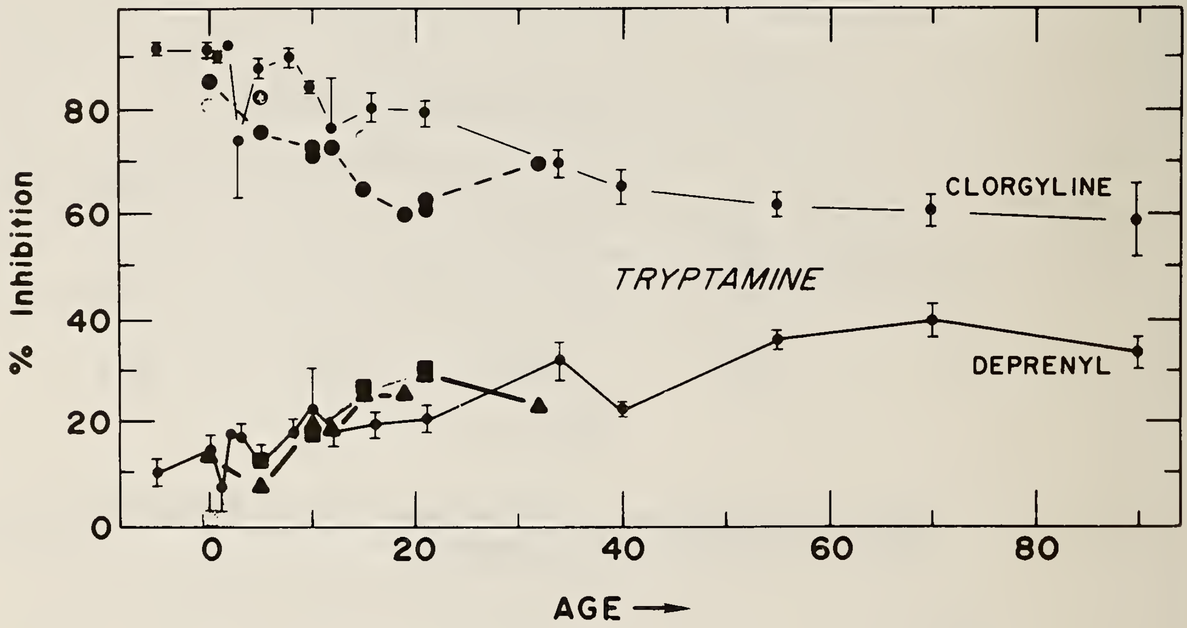


Figure 6. Same as Figure 4 Except That PEA Was the Substrate

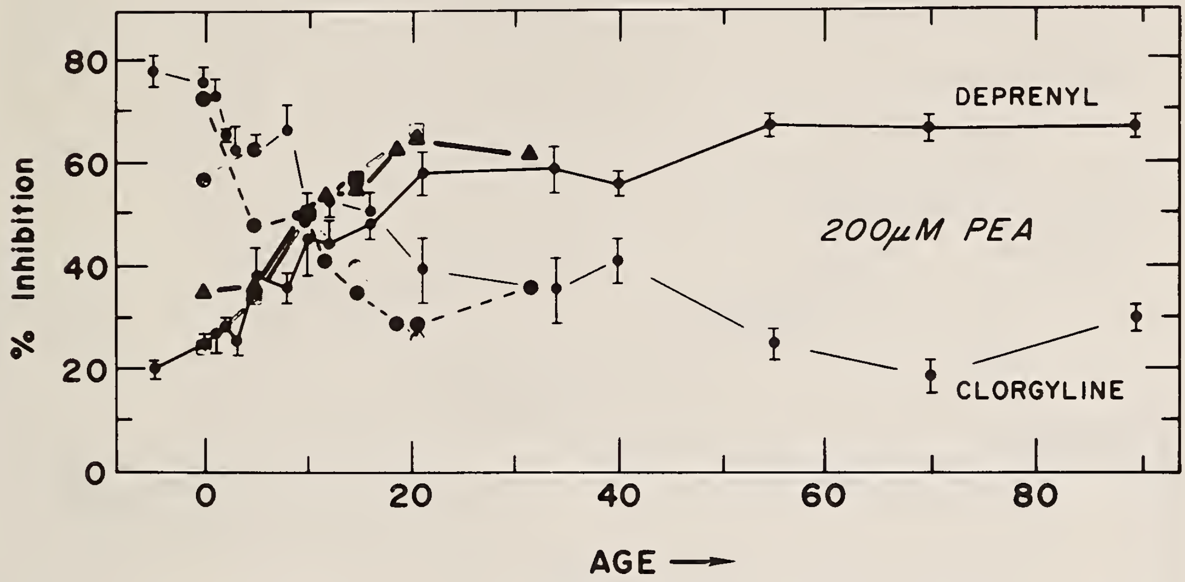


Figure 7. Specific Activity of MAO in 40-Day-Old Normal Rat Brain at Varying Concentrations of Substrate (PEA)

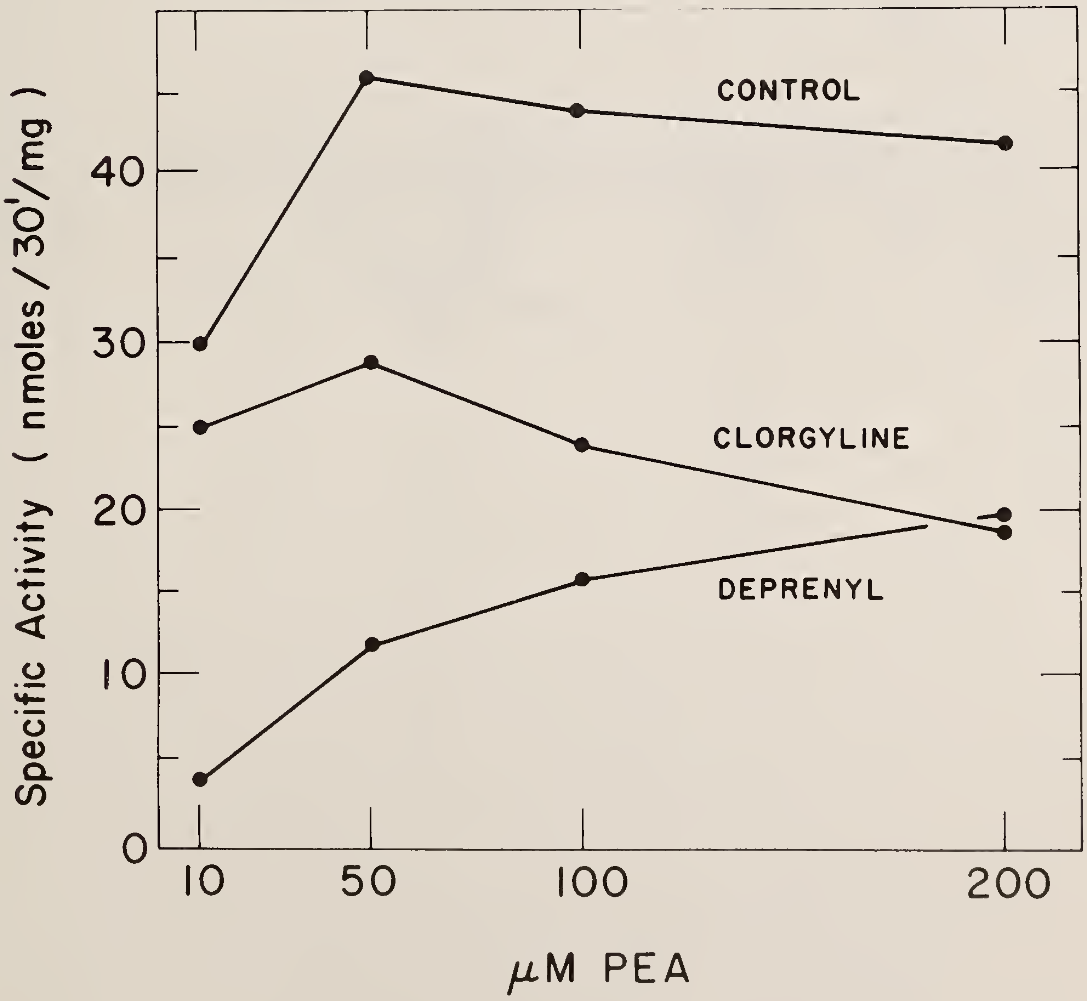


Figure 8. Sequential Inhibition of MAO in 40-Day-Old Rat Brain With PEA as Substrate

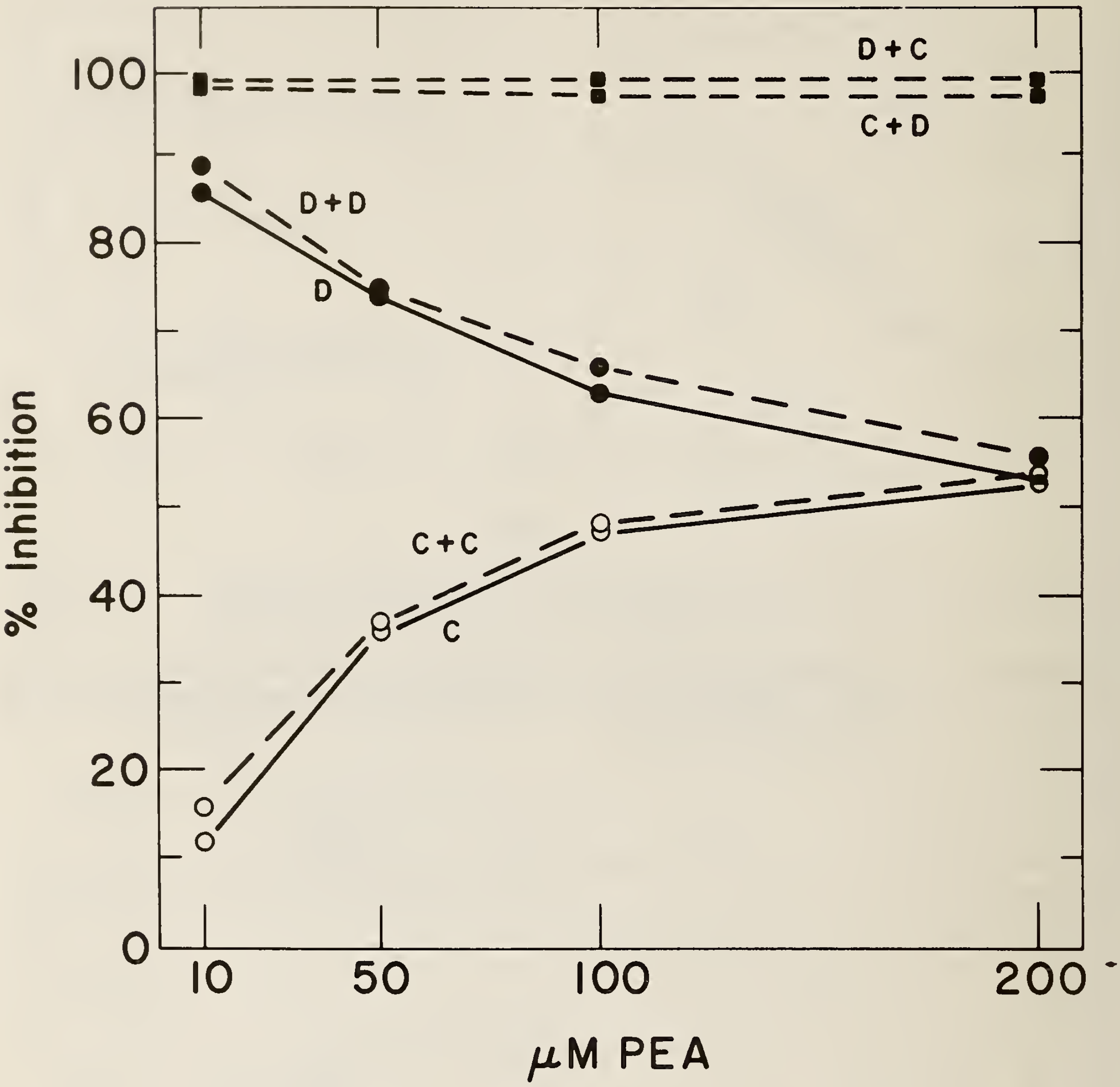


Figure 9. Inhibition of MAO Over Different Ages With Varying Concentrations of PEA

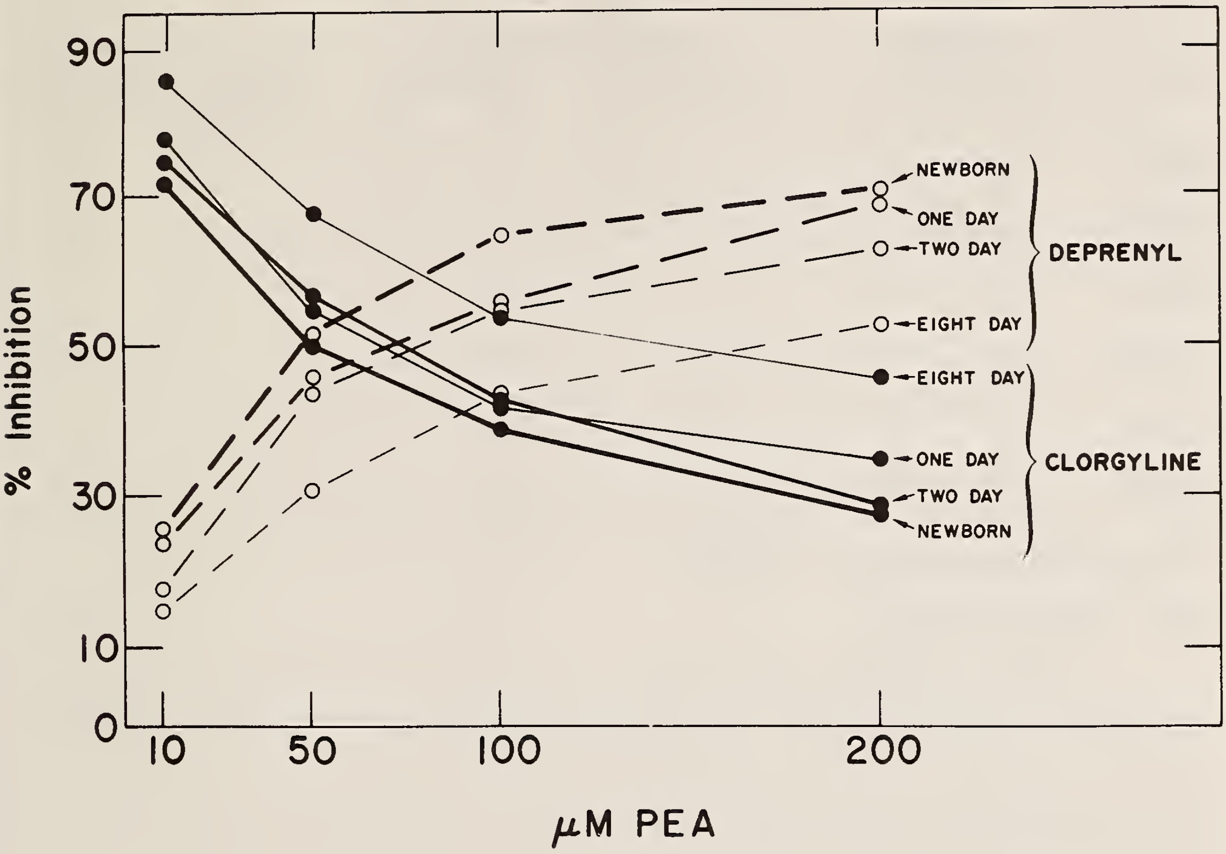


Table 1. Inhibition of MAO in 40-Day-Old Normal Rat Brain by Clorgyline and Deprenyl

µM PEA	Control Expt'l.	 Calculated	Blocked w/ Deprenyl	Blocked w/ Clorgyline
10	30	29	4	25
50	46	41	12	29
100	44	40	16	24
200	42	39	20	19

Table 2. Comparison of Synaptosomal and Whole Brain Mitochondrial MAO

	Specific Activity Control		Percent Inhibition				With Oxygen Percent Activation	
			Deprenyl		Clorgyline			
	Mito[a]	Syn[b]	Mito	Syn	Mito	Syn	Mito	Syn
Normal	33	21	30	11	47	52	24	0
Alcohol	29	25	28	8	40	55	3	0
Normal	38	13	73	38	26	55	40	41
Alcohol	25	14	70	41	30	51	48	37
Normal	40	30	6	3	93	93	0	0
Alcohol	33	36	6	5	91	90	6	7

[a] Whole brain mitochondrial MAO.

[b] Synaptosomal MAO.

References

Branchey, L., and Friedhoff, A.J. The influence of ethanol administered to pregnant rats on tyrosine hydroxylase activity of their offspring. *Psychopharmacology*, 32:151-156, 1973.

Chweh, A.Y., and Leslie, S.W. Effects of acute and chronic administration of ethanol on rat brain arylsulphatase A and B. *Life Sci*, 27:1777-1782, 1980.

Cook, T.M.; Komiskey, H.L.; and Hayton, W.L. The influence of ethanol withdrawal on the binding site of 3H-dopamine. *Proc West Pharmacol Soc*, 23:157-158, 1980.

Dinovo, E.C.; Grieber, B.; and Noble, E.P. Alterations of fast-reacting sulfhydryl groups of rat brain microsomes by ethanol. *Biochem Biophys Res Commun*, 68:975-981, 1976.

Eiduson, S., and Buckman, T. Studies of MAO using spin labeled probes. In: Singer, T.P.; Von Korff, R.W.; and Murphy, D.L., eds. *Monoamine Oxidase: Structure, Function and Altered Functions*. New York: Academic Press, 1979. pp. 213-231.

Gursey, D., and Olson, R. Depression of serotonin and norepinephrine levels in brain stem of rabbit by ethanol. *Proc Soc Exp Biol Med*, 104:280-281, 1960.

Karwaka, H.; Sitkiewicz, D.; Skonieczna, M.; Bricz, W.; and Dymecki, J. Ultrastructural and biochemical studies of the brain and other organs in rat after chronic ethanol administration. III. Influence of ethanol intoxication on oxidative phosphorylation of the rat brain mitochondria with ultrastructural and morphometric evaluation of mitochondrial fraction. *Exp Pathol* (Jena), 18:181-192, 1980.

Kumar, S., and Rawat, A.K. Prolonged ethanol consumption by pregnant and lactating rats and its effect on cerebral dopamine in the fetus and the neonate. *Res Commun Psychol Psychiatry Behav*, 22:259-277, 1977.

Kurosawa, Y. Studies on monoamine oxidase (Report XXV). Effects of alcohols on beef liver mitochondrial monoamine oxidase. *Jpn J Pharmacol*, 24:787-795, 1974.

Lau, C.; Thadani, P.V.; Schanberg, S.M.; and Slotkin, T.A. Effects of maternal ethanol ingestion on development of adrenal catecholamines and dopamine-β-hydroxylase in the offspring. *Neuropharmacology*, 15:505-507, 1976.

Littleton, J.M.; Grieve, S.J.; Griffiths, P.J.; and John, G.R. Ethanol-induced alteration in membrane phospholipid composition: Possible relationship to development of cellular tolerance to ethanol. *Adv Exp Med Biol*, 126:7-19, 1980.

Masamoto, K.; Arai, Y.; Kuroiwa, Y.; Kurosawa, Y.; and Yashuhara, H. Studies on monoamine oxidase (Report 26). *Folia Pharmacol Jpn*, 70:747-756, 1974.

Maynard, L., and Schenker, V. Monoamine oxidase inhibition by ethanol in vitro. *Nature*, 196:575-576, 1962.

Rawat, A.K. Developmental changes in the brain levels of neurotransmitters as influenced by maternal ethanol consumption in the rat. *Neurochem*, 28:1175-1182, 1977.

Reitz, R.C. Effect of chronic ethanol ingestion on membrane lipids. *Proc West Pharmacol Soc*, 23:437-440, 1980.

Renis, M.; Giovini, A.; and Bertolino, A. Variazioni Indotte Dall'Etanolo

Sull'Attivita Monoaminoosidasica Di Mitocondri Di Cervello Di Ratto: MAO-Da E MAO-NA. *Acta Neurol*, 29:293-298, 1974.

Reyes, E. The effects of alcohol on gamma-glutamyl transpeptidase: A membrane bound enzyme. *Proc West Pharmacol Soc*, 23:433-436, 1980.

Rosenfeld, G. Inhibitory influence of ethanol on serotonin metabolism. *Proc Soc Exp Biol Med*, 103:144-149, 1960.

Seeman, P., and Roth, S. General anesthetics expand cell membranes at surgical concentrations. *Biochim Biophys Acta*, 255:171-177, 1972.

Shih, J.C., and Eiduson, S. Multiple forms of monoamine oxidase in developing brain: Tissue and substrate specificities. *J Neurochem*, 18:1221-1227, 1973.

Shih, J.C., and Eiduson, S. Monoamine oxidase (EC 1.4.3.4): Isolation and characterization of multiple forms of the brain enzyme. *J Neurochem*, 21:41-49, 1973.

Streissguth, A.P. Fetal alcohol syndrome: An epidemiologic perspective. *Am J Epidemiol*, 107:467-478, 1978.

Tabakoff, B. Neurochemical aspects of ethanol dependence. In: Blum, K., ed. *Alcohol and Opiates, Neurochemical and Behavioral Mechanisms*. New York: Academic Press, 1977. pp. 21-40.

Taylor, A.N.; Branch, B.J.; Liu, S.H.; Wiechman, A.F.; Hill, M.A.; and Kokka, N. Fetal exposure to ethanol enhances pituitary-adrenal and temperature responses to ethanol in adult rats. *Alcohol Clin Exp Res*, in press.

Thadani, P.V.; Lau, C.; Slotkin, T.A.; and Schanberg, S.M. Effects of maternal ethanol ingestion on amine uptake into synaptosomes of fetal and neonatal rat brain. *J Pharmacol Exp Ther*, 200:292-297, 1977.

Towne, J.C. Effect of ethanol and acetaldehyde on liver and brain monoamine oxidase. *Nature*, 201:709-710, 1964.

Walsh, M.; Truitt, E., Jr.; and Davis, V. Acetaldehyde mediation in the mechanisms of ethanol-induced changes in norepinephrine metabolism. *Mol Pharmacol*, 6:416-424, 1970.

Yanai, J.; Sze, P.Y.; and Ginsburg, B.E. Effects of aminergic drugs and glutamic acid on audiogenic seizures induced by early exposure to ethanol. *Epilepsia*, 16:67-71, 1975.

Summary

Victor M. Hesselbrock, Edward G. Shaskan, and Roger E. Meyer

Studies of alcoholism, like studies of other chronic diseases, are beginning to focus on factors relevant to host susceptibility in addition to environmental influences, symptoms of the disease, and biological or social consequences of long-term drinking behavior. In short, studies of alcoholism are beginning to seriously consider the tenets of chronic disease epidemiology, and the associated new field of genetic epidemiology (Morton and Chung 1978; Sing and Skolnick 1979) is gaining widespread theoretical acceptance.

It is becoming increasingly clear that biologic and genetic heterogeneity are too frequently unrecognized in psychiatric nosology (Buchsbaum and Rieder 1979; Goodwin and Guze 1979; Rieder and Gershon 1978). Further, psychiatric diagnostic criteria (including DSM III) are often multiaxial descriptions of aberrant personality and symptom formations, but rarely provide insight into etiologies. Notwithstanding the courageous, and frequently tedious, attempts to develop treatment-relevant typologies for alcoholism (Meyer and Mirkin in press; National Institute on Alcohol Abuse and Alcoholism 1981), it is evident that these classifications also are confounded by consequences of the disease, especially since psychosocial and biological data are usually collected (no matter how carefully and devotedly) retrospectively.

With the exception of existing population studies designed originally for other purposes (Vaillant 1979), fully prospective epidemiologic studies of theoretically relevant biologic and psychosocial risk factors have not been attempted in the study of alcoholism. Frankly, even at this stage of our knowledge of biological and psychosocial concomitants of alcoholism, we must ask whether such a fully prospective population study of alcoholism would be theoretically warranted or cost effective.

Accordingly, what kinds of studies can be designed to begin the arduous task of sorting out antecedent factors in the pathogenesis of alcoholism? How can we effectively distinguish the biologic and genetic factors related to host vulnerability to alcoholism from the biologic, psychosocial, and (even?) genetic consequences of long-term ethanol abuse? One has only to consider the pharmacologic distribution of this relatively nonpolar, low molecular weight chemical throughout the body's organs and tissues to appreciate the complexity of this seemingly simple question. Thus, in 1981, it is still being argued by experimentalists of organismic and cellular pharmacology whether ethanol itself or its polar and highly toxic product, acetaldehyde, is the agent through which the pharmacological tolerance and physical dependence develop.

The purpose of this symposium, sponsored by NIAAA and ACNP, was to bring together experts in the genetic epidemiology of alcoholism and experts in the studies of two purported biologic risk factors in the etiology of alcoholism, acetaldehyde (AcH) and monoamine oxidase (MAO). Although other biologically relevant risk factors for alcoholism were excluded from a formal treatment in this symposium, this omission should not suggest judgmental bias by the organizers. Indeed, a symposium to discuss only biologic risk factors for alcoholism would easily take several days. For example, important genetic issues related to isoenzymes of aldehyde dehydrogenase (Koivula and Koivusalo 1975; Marjanen 1973), although necessarily included in the texts of various speakers, were not specifically addressed at this symposium.

The exclusion of relevant and worthwhile topics was necessary in order to accomplish the task of bringing together genetic epidemiologists and biochemical neuropsychopharmacologists so that they could present and discuss the major theoretical, methodological, and logistical issues associated with investigating possible biological pathogenetic mechanisms of alcoholism.

To the reader of this symposium, we apologize for the absence of transcribed discussions of the proceedings. Indeed, it is a tribute to the rules and philosophy of the ACNP that tape recordings of presentations are disallowed and quotations from presentations at these meetings are limited by the express permission of the secretariat. The symposium in San Juan, under the same name as this volume, was an all-day affair and the spirit of collegiality and professionalism was rampant. In the words of one senior ACNP member, ". . .it recollected the 'good old days at ACNP' and epitomized the concept of a workshop." We hope that this volume and our efforts at summarizing presentations and discussions lend some verisimilitude to the spirit of that event.

The underpinning genetic structure of the symposium was defined by the St. Louis group. Rice and coworkers reviewed some precepts of genetic epidemiology and then suggested ways in which family studies can be used to investigate traits that have high familial resemblance, but for which interactions of biological and psychosocial risk factors are not well understood. Building upon threshold models in human genetics and using the simple familial trait "tall" as an example, Rice and coworkers lucidly derived mathematical models that have relevance to the more complex approaches of path analysis and segregation analysis. Future directions of this type of modeling for the purpose of predicting risks and identifying biological or environmental factors were discussed.

In a thorough and scholarly review of the biochemistry and pharmacology of acetaldehyde, Schuckit integrated his pioneering studies on blood levels of AcH in family history negative and positive for alcoholism subjects. After reviewing the methodological difficulties in measuring blood AcH levels (a complex subject in itself), Schuckit shared a replication of his earlier published report of increased AcH in blood of family history positive subjects following consumption of ethanol under controlled laboratory conditions (Schuckit and Rayses 1979). To his credit, and to the benefit of this symposium and volume, Schuckit clearly assessed the pitfalls of his own experimental design and provided suggestions for correcting laboratory and selection bias.

On a more experimental basis, the papers presented by Lieber, Dannecker, and Petersen focused more upon the state-of-the-art acetaldehyde evaluations in primates, including man, and in genetically distinct strains of mice. From this perspective, it was felt that the more genetically oriented participants benefited from the presentations and discussion in the same way that the biochemical pharmacologists had earlier learned from the geneticists. Lieber presented a concise cellular basis for his experimental assessments of ethanol and acetaldehyde metabolism in humans and baboons. His attention to kinetic principles of intermediary metabolism and biological ultrastructure provides the basic framework needed to justify compartmental evaluations of AcH in both blood and breath at the organismic level. Given this background, we can then appreciate Lieber's assessments of the relevance of acetaldehyde in the Oriental "facial flush syndrome," as well as the hotly debated relevance of TIQs in the development of tolerance and physical dependence to ethanol.

We were treated to a novel experimental approach for the evaluation of breath acetaldehyde and other potentially toxic volatile components identified in human breath. Because this new technique, which measures nondrinking (i.e., endogenous) levels of ethanol, acetaldehyde, and methanol, represented efforts by new workers in the field (Dannecker and associates), it was reassuring to note that the identified confounding factor of cigarette smoking was also carefully addressed by Lieber and coworkers. Thus, both groups independently identified a major confounding factor not only of breath acetaldehyde measurement but also of broader pathogenic significance—namely, cigarette smoking. With appropriate attention to both laboratory and epidemiologic design pitfalls, the methodological advantages of the new breath acetaldehyde procedure described by Dannecker and coworkers suggests high utility, noninvasive population studies along pedigree or family lines.

Petersen and coworkers then raised the important question of the amount of acetaldehyde needed in brain in order to markedly influence behavior. They were able to document genetic influences on the disposition of AcH in the mouse brain utilizing DBA-2 and C57BL/6 mice. These authors also discussed the interaction of environmental influences (diet) on the disposition of acetaldehyde and ethanol and identified issues related to possible genetic predisposition to toxic manifestations of ethanol consumption in mouse and humans. Accordingly, the conclusions of Petersen and coworkers clearly focused on the need for more studies of the relationship between genetic and environmental risk factors.

Until recently one of the most replicated findings among populations diagnosed with alcoholism has also been the most problematic in understanding its role in the pathogenesis of alcoholism. We refer to the almost universal finding of low platelet MAO activity among diverse populations diagnosed with alcoholism as compared with other control populations. For instance, in contrast to the situation for schizophrenia (Wyatt et al. 1978), Oreland's assessment of the world literature could not find a single report in which a "normal" mean activity for MAO was found in subjects diagnosed with alcoholism. This statement by Oreland, who was the only invited participant from outside the continental

United States, is also reflected in table 1 of Shaskan's review of platelet MAO activity as a risk factor. Therefore, an ascertainment bias cannot be attributed solely to cross-cultural factors even at the level of the Arctic Circle (Oreland is from Umea, Sweden). Oreland's question, "Why do alcoholics have low platelet monoamine activity?" is seminal to this portion of the symposium. His review of genetic control of MAO activity, the types of MAO, and their kinetic properties forms the basic science approach so relevant to assessments attempting to answer this question. Through a careful review of his own work with Gottfries, Wiberg, and others on alcoholism and other diseases of suspected chronic etiology, Oreland reflected on the possible etiologic significance of a constitutionally "weak" brain monoaminergic system. He supported such speculation with his own group's experimental evidence and a recent finding from Dennis Murphy's laboratory in the United States that suggests a common ontogenetic origin of human thrombocytes (blood platelets) and brain tissue (Marangos et al. 1980).

Constitutional factors are risk factors in epidemiologic parlance, and Shaskan reviewed the possible role of MAO activity, citing some of his own unpublished data, in the framework of precepts of chronic disease epidemiology. Shaskan's data comparing admission platelet enzyme activity from patients hospitalized with alcoholism with their 1-year followup visit did not adequately distinguish low MAO activity as an antecedent risk factor from low MAO activity as a consequence of sustained ethanol consumption. This example of a potentially relevant factor for the pathogenesis of alcoholism that can be confounded by environmental factors serves as a reminder of the most common methodologic pitfall in case–control studies of chronic disease.

An additional relevant issue for chronic disease epidemiology is the possibility of an interaction between antecedent biologic risk factors and outcome factors of the disease process resulting in biologically identifiable typologies. For instance, Lieber and coworkers presented a schema by which chronic ethanol consumption induces a "vicious circle" at the cellular level, resulting in progressive mitochondrial impairment (Lieber et al. this volume, figure 7). Since MAO is a mitochondrial enzyme, its impairment is likely in the schema of Lieber and coworkers, but conceivably only in those individuals biologically or otherwise predisposed to mitochondrial or cellular damage.

Sullivan and coworkers reviewed a wide range of metabolic factors in humans that can influence MAO activity and, using a diagnosis of secondary depression as a psychobiological outcome of alcoholism, prospectively evaluated stable baseline MAO values as predictors of response to lithium treatment. In their well-designed study, neither a placebo nor lithium resulted in significant improvement in depression among the alcoholic cohort with low platelet MAO activity. A significant improvement, as measured by standard depression rating scales, was found among the lithium-treated, high MAO cohort of patients diagnosed with primary alcoholism. In his characteristically insightful manner, Sullivan discussed the results of his findings in the context of the relationship between alcoholism and affective illness, recalling the editors' admonition in the introduction of this volume that biologic and genetic factors may also reflect a general predisposition to psychopathology.

Certainly of relevance is the possibility that irreversible ontogenetic changes may also occur among biologic entities before birth. In populations in which the use and abuse of ethanol and other congeners by pregnant women may precipitate clinical and subclinical changes reflected as the fetal alcohol syndrome (FAS), the design of family studies for identification of possible biologic risk factors needs to consider possible prenatal influences, lest putative biologic vulnerability factors be again confounded as outcome factors from prenatal pathogenesis. Although Eiduson and Groshong carefully qualify the universal validity of their rat brain model for FAS, their thorough study of both forms of brain MAO in this animal model suggests that monoamine oxidase activity is not appreciably altered by ingestion of alcohol by the dams of newborn rats.

Most of the participants in this symposium either received their training in clinical sciences or spend large proportions of their time and effort with persons and issues of clinical relevance. Indeed, as clinicians and basic scientists in modern postindustrialized societies, we are all too frequently reminded of the clinical effects of ethanol on ourselves, relatives, and friends. However, the focus of the clinician upon the individual with a diagnosis of alcoholism often excludes from view the vast majority of the population who consume ethanol (often in excess) but who will never receive a diagnosis of alcoholism. The bench scientist, who need not be obsessed with the daily rigors of clinical medicine, is obsessed in his own way about methods for improving the replicability of experimental tools and shudders from the thought of variability. However, variability through natural selection is the very basis of the dichotomous distinction between health and disease as required by threshold observations. The multifactorial components leading to such a threshold indicator can never be observed in the individual upon whom natural selection is dependent, but only at the level of the population. Interestingly, the smallest unit of population biology is the family, and compelling reasons for familial studies of alcoholism have been presented at this symposium. Biology and medicine must remember that the scientific principles of epidemiology, like the principles of population genetics and evolution, do not predict for the individual but for the population. The uncertainty of our predictions should not depress our will to predict and thereby alter the course of disease, but force us to strive to improve our predictions and to live with the certainty of uncertainty.

References

Bosron, W.F.; Li, T-K.; and Vallee, B.L. New molecular forms of human liver alcohol dehydrogenase: Isolation and characterization of ADH. *Proc Natl Acad Sci USA*, 77:5784-5788, 1980.

Buchsbaum, M.S., and Rieder, R.O. Biologic heterogeneity and psychiatric research—platelet MAO activity as a case study. *Arch Gen Psychiatry*, 36:1163-1169, 1979.

Goodwin, D., and Guze, S. *Psychiatric Diagnosis*. New York: Oxford University Press, 1979.

Koivula, T., and Koivusalo, M. Different forms of rat liver aldehyde dehydrogenase and their subcellular distribution. *Biochim Biophys Acta*, 397:9-23, 1975.

Marangos, P.J.; Campbell, I.C.; Schmechel, D.E.; Murphy, D.L.; and Goodwin, F.K. Blood platelets contain a neuron-specific enolase subunit. *J Neurochem*, 34:1254-1258, 1980.

Marjanen, L. Comparison of aldehyde dehydrogenases from cytosol and mitochondria of rat liver. *Biochim Biophys Acta*, 327:238-246, 1973.

Meyer, R.E., and Mirkin, P. Typologies in alcoholism: An overview. *Int J Addict*, in press.

Morton, N.E., and Chung, C.S. *Genetic Epidemiology*. New York: Academic Press, 1978.

National Institute on Alcohol Abuse and Alcoholism. *Evaluation of the Alcoholic*, Meyer, R.E., ed. NIAAA Research Monograph No. 5. DHHS Pub. No. (ADM)81-1033. Washington, D.C.: Supt. of Docs., U.S. Govt. Print. Off., 1981.

Rieder, R.O., and Gershon, E. Genetic strategies in biological psychiatry. *Arch Gen Psychiatry*, 35:866-873, 1978.

Schuckit, M., and Rayses, V. Ethanol ingestion: Differences in blood acetaldehyde concentrations in relatives of alcoholics and controls. *Science*, 203:54-55, 1979.

Sing, C., and Skolnick, M. *Genetic Analysis of Common Diseases: Application to Predictive Factors in Coronary Disease.* New York: Alan Liss, 1979.

Vaillant, G.E. Natural history of male psychologic health. *N Engl J Med*, 23:1249-1254, 1979.

Wyatt, R.J.; Potkin, S.G.; Bridge, T.P.; Friedl, W.; and Vogel, F. A twin study on three enzymes (DBH, COMT, MAO) of catecholamine metabolism. Correlations with MMPI. *Psychopharmacology*, 57:63-69, 1978.

DHHS Publication No. (ADM) 83-1199
Printed 1983

3 3 3 0

NIH LIBRARY
Amazing Research.
Amazing Help.

http://nihlibrary.nih.gov

10 Center Drive
Bethesda, MD 20892-1150
301-496-1080

WERT
BOOKBINDING
Grantville, Pa.
Jan — Feb 1985
We're Quality Bound

NIH LIBRARY
3 1496 01022 8024